FORMULATION AND EVALUATION OF TRANSDERMAL PATCH OF METHANOLIC EXTRACT OF *Acalypha indica* Linn. USING DIFFERENT POLYMERS

Dissertation submitted to

The Tamilnadu Dr.M.G.R.Medical University
Chennai – 600 032

In partial fulfillment for the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

By

Reg.No: 261210202

DEPARTMENT OF PHARMACEUTICS

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APRIL - 2014

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ABBREVATIONS

^{o}C	Degree Celsius
Cm^2	Square centimeter
SC	Stratum corneum
HPMC	Hydroxy Propy [Methyl cellulose
SLS	Sodium Lauryl Sulphate
TDDS	Transdermal Drug Delivery System
FT-IR	Fourier Transform Infrared Spectroscopy
e. g.	Example
Sq.ft	Square feet
Rpm	Revolution per minute
ml	Milliliter
nm	Nanometer
RH	Relative Humidity
hrs	Hours
MU	Moisture Uptake
SD	Standard deviation
MC	Moisture Content
IP	Indian Pharmacopoeia
ME	Methanolic Extract
MEA	Methanolic Extract of *Acalypha indica* Linn.

sec	Seconds
kg	Kilogram
gm	gram
SR	Sustained release
PB	Phosphate Buffer
Fig	Figure

1. INTRODUCTION

1.1. Traditional System of Medicines[1, 2, 3]

India has a rich heritage of traditional medicine and the traditional health care system have been flourishing for many centuries. traditional medicine, defined by the WHO as "medical knowledge systems that developed over generations within various societies before the era of modern medicine, including the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being" is used globally and has rapidly growing economic importance. In developing countries, traditional medicine is often the only accessible and affordable treatment available. In Latin America, the WHO regional office for the Americas (AMRO/PAHO) reports that 71% of the population in China and 40% of the population in Columbia has used traditional medicine.

In many Asian countries traditional medicine is widely used, even though western medicine is often readily available. In Japan, 60-70% of allopathic doctors prescribe traditional medicines for their patients. In the US the number of visits to providers of Complementary Alternative Medicine (CAM, codified herbal medicine) now exceeds by far the number of visits to all primary care physicians.

Ayurveda

Ayurvedic medicine is a system of traditional medicine native to the Indian subcontinent and practiced in other parts of the world as a form of traditional medicine. In Sanskrit, the words ayurveda consist of the word say us, means "longevity", and veda, means "related to knowledge" or "science". Evolving throughout its history, ayurveda remains an influential system of medicine in South Asia. The earliest literature on Indian medical practice appeared during the vedic period in India. The Susruta Samhta-and the Charaka Samhta- where influential works on traditional medicine during this era. Ayurveda is considered to be a form of complementary and

alternative medicine (CAM) in the western world, where several of its methods, such as the use of herbs, massage, and yoga, are applied on their own as a form of CAM treatment.

Ayurveda is the ancient (before 2500 B.C.) Indian system of health care involving a holistic view of man, his health, and illness. Ayurvedic treatment of a disease consists of salubrious use of drugs, diets, and certain practices. Medicinal preparation is invariably complex mixtures, based mostly on plant products. Around 1,250 plants are used in various ayurvedic preparations.

Many Indian medicinal plants have come under scientific scrutiny since the middle of the nineteenth century, although in a sporadic fashion.

The first significant contribution from ayurvedic material medica came with the isolation of the hypertensive alkaloids from the sarpagandha plant (*Rauwolfia serpentina*), valued in ayurveda for the treatment of hypertension, insomnia and insanity. This was the first important ancient-modern concordance in ayurvedic plants.

According to ayurveda, all objects in the universe including human body are composed of five basic elements (Panchamahabhutas) namely, earth, water, fire, air and vaccuum (ether). There is a balanced condensation of these elements in different proportions to suit the needs and requirements of different structures and functions of the body matrix and its parts. The growth and development of the body matrix depends on its nutrition, i.e. on food. The food, in turn, is composed of the above five elements, which replenish or nourish the like elements of the body after the action of bio-fire (agni). The tissues of the body are the structural whereas humours are physiological entities, derived from different combinations and permutations of Panchamahabhutas. Treatment of the disease consists in avoiding causative factors responsible for disequilibrium of the body matrix or of any of its constituent parts through the use of Panchkarma procedures, medicines, suitable diet, activity and regimen for restoring the balance and strengthening the body mechanisms to prevent or minimize future occurrence of the disease.

Use of these three measures is done in two ways. In one approach of treatment the three measures antagonize the disease by counteracting the etiological factors and various manifestations of the disease.

In the second approach the same three measures of medicine, diet and activity are targeted to exert effects similar to the etiological factors and manifestations of the disease process. These two types of therapeutic approaches are respectively known as Vipreeta and Vipreetarthkari treatments.

Siddha

Siddha system is one of the oldest systems of medicine in India. The term siddha means achievements and siddhars were saintly persons who achieved results in medicine. Eighteen Siddhars were said to have contributed towards the development of this medical system. Siddha literature is in tamiland it is practiced largely in tamil speaking part of India and abroad. The siddha system is largely therapeutic in nature.

This principles and doctrines of this system, both fundamental and applied, have a close similarity to ayurveda, with specialization in iatro-chemistry. According to this system the human body is the replica of the universe and so are the food and drugs irrespective of their origin. Like ayurveda, this system believes that all objects in the universe including human body are composed of five basic elements namely, earth, water, fire, air and sky. The food, which the human body takes and the drugs it uses are all, made of these five elements. The proportion of the elements present in the drugs vary and their preponderance or otherwise is responsible for certain actions and therapeutic results. As in ayurveda, this system also considers the human body as a conglomeration of three humours, seven basic tissues and the waste products of the body such as faeces, urine and sweat. The food is considered to be basic building material of human body which gets processed into humours, body tissues and waste products. The equilibrium of humours is considered as health and its disturbance or imbalance leads to disease or sickness. This system also deals with the concept of salvation in life. The exponents of this system consider achievement of this state is possible by medicines and meditation.

The siddha system is capable of treating all types of disease other than emergency cases. In general, this system is effective in treating all types of skin problems particularly psoriasis, STD, urinary tract infections, diseases of liver and gastro intestinal tract, general debility, postpartum anemia, diarrhoea and general fevers.

Unani

Unani system of medicines originated in Greece and is based on the teachings of Hippocrates and Gallen and it developed in to an elaborate medical system by Arabs, like Rhazes, Avicenna, Al-Zahravi, Ibne-Nafis and others. Unani medicines got enriched by imbibing what was best in the contemporary systems of traditional medicines in Egypt, Syria, Iraq, Persia, India, China and other Middle East countries. In India, unani system of medicine was introduced by Arabs and soon it took firm roots. The Delhi Sultans (rulers) provided patronage to the scholars of unani system and even enrolled some as state employees and court physicians. During 13th and 17th century A.D. Unani medicine had its hey-day in India. During the British rule, unani system suffered a setback due to withdrawal of State Patronage, but continued to be practiced as the masses reposed faith in the system. It was mainly Sharif family in Delhi, the Azizi family in Lucknow and the Nizam of Hyderabad due to whose efforts unani medicine survived during the British period. In India, the concept of research in unani system of medicine was originally perceived by Masih-ul-Mulk Hakim Ajmal Khan in the 1920s. A versatile genius of his time, Hakim Ajmal Khan spotted Dr. Salimuzzaman Siddiqui- a chemist- for undertaking chemical studies on some important medicinal plants used in unani medicine. Dr. Siddiqui undertook the task visualized by Masih-ul-Mulk and his discovery of medicinal properties of a plant, commonly known as Asrol (*Pagalbooti*), led to sustained research that established the unique efficacy of this plant known all over the world as *Rauwolfia serpentina*, in neurovascular and nervous disorders, such as hypertension, insanity, schizophrenia, hysteria, insomnia and psychosomatic conditions, *etc*.,

Homoepathy

Homeopathy, founded by a German physician Samuel Hahnemann in 1790, is based on the idea that ‘like cures like’; that is substances that cause certain symptoms in a healthy person can also cure those same symptom in someone who is sick. This so called low of similar gives homeopathy its name ‘homeo’ for similar ‘pathy’ designating disease. In this experiment Hahnemann developed a method of ‘potentizing’ homeopathic remedies by diluting them in a water-alcohol solution and then vigorously shaking the mixtures.

The result convinced him that a high degree of dilution not only minimizes the side effects of the remedies but also simultaneously enhances their medical efficacy. Most Homeopathic remedies have undergone ‘proving’ or medical observation in which healthy individuals are given doses of undiluted homeopathic substances.

Mental, emotional, psychic and other details of the patients are most important. This leads the physician to a better understanding of which remedy will best suits a particular set of symptoms. Over the past 200 years, providing for almost 2,000 substances have been conducted.

International diversity

Traditional medicine practices have been adopted in different cultures and regions without the parallel advance of international standards and methods for evaluation.

National policy and regulation

Many countries have not national policies for traditional medicine. Regulating traditional medicine products, practices and practitioners is difficult due to variations in definitions and categorizations of traditional medicine therapies. A single herbal product could be defined as a food, a dietary supplement or an herbal medicine, depending on the country. This disparity in regulations at the national level has implications for international access and distribution of products.

Safety, effectiveness and quality

Scientific evidence from tests done to evaluate the safety and effectiveness of traditional medicine products and practices is limited. While evidence shows that acupuncture, some herbal medicines and some manual therapies (*e.g.* massage) are effective for specific conditions, further study of products and practices is needed. Requirements and methods for research and evaluation are complex. For example, it can be difficult to assess the quality of finished herbal products. The safety, effectiveness and quality of finished herbal medicine products depend on the quality of their source materials (which can include hundreds of natural constituents), and how elements are handled through production processes.

Knowledge and sustainability

The expanding herbal product market could drive over-harvesting of plants and threaten biodiversity. Poorly managed collection and cultivation practices could lead to the extinction of endangered plant species and the destruction of natural resources. Efforts to preserve both plant populations and knowledge on how to use them for medicinal purposes is needed to sustain traditional medicine.

Patient safety and use

Many people believe that because medicines are herbal (natural) or traditional they are safe (or carry no risk for harm). However, traditional medicines and practices can cause harmful, adverse reactions if the product or therapy is of poor quality, or it is taken in appropriately or in conjunction with other medicines. Increased patient awareness about safe usage is important, as well as more training, collaboration and communication among providers of traditional and other medicines.

1.2. Novel Drug Delivery System[4]

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce

no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology.

To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among drug carriers one can name soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins,liposomes and micelles. The carriers can be made slowly degradable, stimuli-reactive (*e.g.*, pH- or temperature-sensitive), and even targeted (e.g., by conjugating them with specific antibodies against certain characteristic components of the area of interest). Targeting is the ability to direct the drug-loaded system to the site of interest. Two major mechanisms can be distinguished for addressing the desired sites for drug release: (i) passive and (ii) active targeting. An example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumors as a result of the enhanced vascular permeability of tumor tissues compared with healthy tissue. A strategy that could allow active targeting involves the surface fictionalization of drug carriers with ligands that are selectively recognized by receptors on the surface of the cells of interest. Since ligand–receptor interactions can be highly selective, this could allow a more precise targeting of the site of interest.

Controlled drug release and subsequent biodegradation are important for developing successful formulations. Potential release mechanisms involve: (i) desorption of surface-bound /adsorbed drugs; (ii) diffusion through the carrier matrix; (iii) diffusion (in the case of nanocapsules) through the carrier wall; (iv) carrier matrix erosion; and (v) a combined erosion /diffusion process. The mode of delivery can be the

difference between a drug's success and failure, as the choice of a drug is often influenced by the way the medicine is administered. Sustained (or continuous) release of a drug involves polymers that release the drug at a controlled rate due to diffusion out of the polymer or by degradation of the polymer over time. Pulsatile release is often the preferred method of drug delivery, as it closely mimics the way by which the body naturally produces hormones such as insulin. It is achieved by using drug-carrying polymers that respond to specific stimuli (*e.g*., exposure to light, changes in pH or temperature).

For over 20 years, researchers have appreciated the potential benefits of nanotechnologyin vast providing improvement in drug delivery and drug targeting.

Improving delivery techniques that minimize toxicity and improve efficacy offers great potential benefits to patients, and opens up new markets for pharmaceutical and drug delivery companies. Other approaches to drug delivery are focused on crossing particular physical barriers, such as the blood brain barrier, in order to better target the drug and improve its effectiveness; or on finding alternative and acceptable routes for the delivery of protein drugs other than via the gastro-intestinal tract, where degradation can occur.

1.2.1. Topical delivery

Over the last decades, the treatment of illness has been accomplished by administrating drugs to human body via various routes namely oral, sublingual, rectal, parental, topical, inhalation etc. Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders (*e.g.* acne) or the cutaneous manifestations of a general disease (*e.g*. psoriasis) with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin. Semi-solid formulation in all their diversity dominate the system for topical delivery, foams, spray, medicated powders, solution, and even medicated adhesive systems are in use.

Topical delivery includes two basic types of product:

- External topical that are spread, sprayed, or otherwise dispersed on to cutaneous tissues to cover the affected area
- Internal topical that are applied to the mucous membrane orally, vaginally or on anorectal tissues for local activity

For the most part topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes. Although some unintended drug absorption may occur, it is sub therapeutics quantities and generally of minor concern.

1.2.2. Advantages of Topical Drug Delivery Systems

- Avoidance of first pass metabolism
- Convenient and easy to apply
- Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time *etc.*,
- Achievement of efficacy with lower total daily dosage of drug by continuous drug input
- Avoids fluctuation in drug levels, inter- and intrapatient variations
- Ability to easily terminate the medications, when needed
- A relatively large area of application in comparison with buccal or nasal cavity
- Ability to deliver drug more selectively to a specific site
- Avoidance of gastro-intestinal incompatibility

1.2.3. Disadvantages of Topical Drug Delivery Systems

- Skin irritation of contact dermatitis may occur due to the drug and/or excipients
- Poor permeability of some drugs through the skin
- Possibility of allergenic reactions

- Can be used only for drugs which require very small plasma concentration for action
- Enzyme in epidermis may denature the drugs

1.3. Transdermal Drug Delivery Systems [5, 6]

The TDDS are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at a controlled rate to the systemic circulation. Transdermal drug delivery is a viable administration route for potent, low-molecular weight therapeutic agents which cannot withstand the hostile environment of gastrointestinal tract and/or subject to considerable first-pass metabolism by the liver.

1.3.1. Advantages of TDDS:

- Transdermal medication delivers a steady infusion of a drug over an extended period of time. Adverse effects or therapeutic failures frequently associated with intermittent dosing can be avoided.
- Transdermal delivery can increase the therapeutic value of many drugs by avoiding specific problems associated with the drug e.g., gastro-intestinal irritation, low absorption, decomposition due to hepatic “first-pass” effect, formation of metabolites that cause side effects, short half-life necessitating frequent dosing etc.
- The simplified medication regimen leads to improved patient compliance and reduced inter-and intra-patient variability.
- Self-administration is possible with these systems.
- The drug input can be terminated at any point of time by removal of drug application from the skin surface.
- Substitute’s oral administration when route is unsuitable as in case of vomiting, diarrhoea and in unconscious patients.
- Achievement of efficacy with lower total daily dosage of drug by continuous drug input.

- Provides utilization of drugs with short biological half-life, narrow therapeutic window.
- Avoids risk and inconveniences of intravenous therapy.
- Reduces the chances of over- or under dosing through the prolonged, preprogrammed delivery of drug at the required therapeutic rate.

1.3.2. Disadvantages of TDDS:

- The drug must have some desirable physicochemical properties for penetration through *Stratum corneum* (SC) and if the drug dosage required for therapeutic value is more than 10 mg/day, the transdermal delivery will be very difficult if not impossible. Daily doses of less than 5mg/day are preferred.
- Skin irritation or contact dermatitis due to the drug, excipients and penetration enhancers used to increase percutaneous absorption of the drug is another limitation.
- Clinical need is another area that has to be examined carefully before a decision is made to develop a transdermal product.
- The barrier function of the skin changes from one site to another on the same person, from person to person and with age.
- Poor permeability of drugs through the skin.
- Enzymes in epidermis may denature the drugs.
- Drugs that require high blood levels cannot be administered.

1.4.The Skin [7,8]

To understand the concept of TDDS, it is important to review the structural and biochemical features of human skin and those characteristics which contribute to the barrier function and the rate of drug access into the body via skin. The skin is one of the most extensive organs of the human body covering an area of about $2m^2$ in an average human adult. This multilayered organ receives approximately one-third of the blood circulating through the body. It has varied functions and properties.

With a thickness of only a millimeter, the skin separates the underlying blood circulation network from the outside environment, serves as a barrier against physical, chemical and microbial attacks, acts as a thermostat in maintaining body temperature, protects against harmful ultraviolet rays of the sum and plays a role in the regulation of blood pressure.

1.4.1. Anatomy of Skin:

The skin is a multi-layered organ and has anatomically many histological layers. The three main layers the skin are epidermis, dermis and hypodermis .

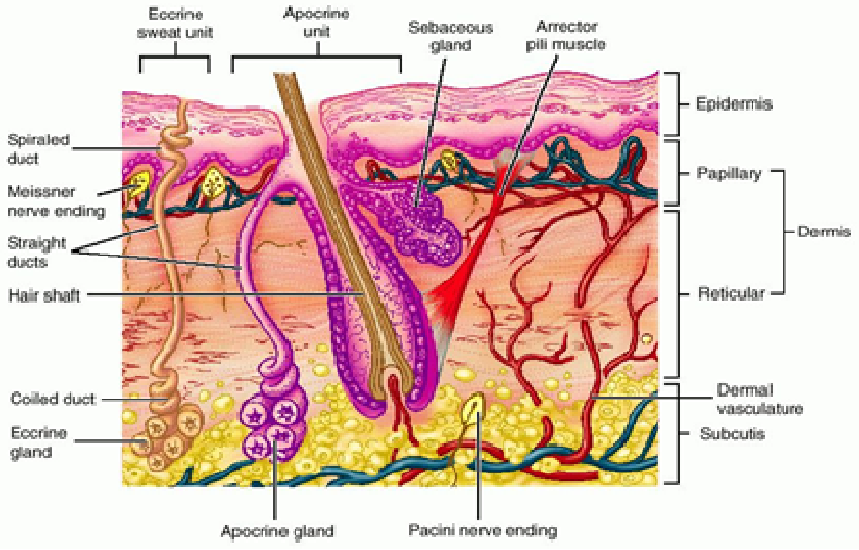


Fig No.1: Anatomy of the Skin

1.4.2. Epidermis:

The epidermis is the outermost layer of the skin; it is approximately 150 micrometer (cum) thick. The SC or non-viable epidermis is the top, outermost layer of the epidermis and is 25-30 layers of horny dead cells, which are flattened, dehydrated and keratinized the interior or these cell layers is crisscrossed with densely packed bundles of keratin fibres.

The dry composition of the horny layer is 75-85% protein; the bulk of the remainder of the substance is a complicated mixture of lipids, this combination of ceratnocytes with interspersed spidermal lipids form a water proof moisture barrier that minimizes trans epidermal water loss (TEWL) to keep moisture in the skin. It is approximately 10-20um thick and acts as protective membrane preventing water loss from the skin and limiting the entry of chemicals from the environment. *Stratum corneum* consists of lipids which are made up of ceramaides and neutral lipids such as free fatty acids free sterols and triglycerides, the remainder is made up of phospholipids, glycosphingolipids and cholesterol sulphate. Below the *Stratum corneum* are the other layers of the epiderm is the *stratum lucidum*, *stratum granulosum*, and stratum spinosum and stratum germinativum, together these layers constitute the viable epidermis.

1.4.3. Dermis:

The dermis is vascularized and the thickest of all the layers (3-5mm thick). It possesses sweat glands, hair follicles, nerve endings and lymph vessels and acts as the systemic absorption site for drugs. The dermis is located between the hypodermis and the epidermis. An average human skin is known to contain on the average 40-70 hair follicle and 200-300 sweat glads on each square cm of skin area.

1.4.4. Hypodermis:

The hypodermis, which is subcutaneous fat layer that functions as insulation and padding for the body. The hypodermis is the deepest section of the skin as shown in the (Fig No.1).

The hypodermis refers to the fat tissue below the dermis that insulates the body from cold temperatures and provides shock absorption. Fat cells of the hypodermis also store nutrients and energy.

1.4.5. Skin Appendages:

The skin is interspersed with hair follicle and associated sebaceous gland like regions two types of sweat glands epicrine and apocrine. Collectively these are referred to as skin appendages.

1.5. Pathway of Transdermal Permeation:

The permeation of drugs through the skin includes the diffusion through the intact epidermis and through the skin appendages, *ie.*, hair follicles and sweat glands, which form shunt pathways through the intact epidermis. However, these skin appendages occupy only 0.1% of the total human skin surface and the contribution of this pathway is usually considered to be small (with only a few exceptions having been noted). As stated above, drug permeation through the skin is usually limited by the *Stratum corneum*. Two pathways through the intact barrier may be identified (Fig.2): the intercellular lipid route between the corneocytes and the transcellular route crossing through the corneocytes and the intervening lipids; that is, in both cases the permeant must diffuse at some point through the intercellular lipid matrix, which is now recognized as the major determinate of percutaneous transport rate.

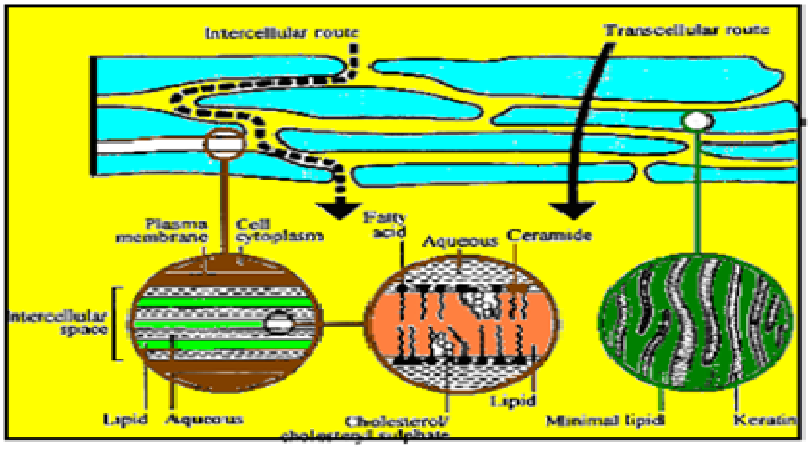


Fig No. 2: Permeation routes through the *Stratum corneum*: (i) via the lipid matrix between the corneocytes (intercellular route) and (ii) across the corneocytes and the intercellular lipid matrix.

1.5. Kinetics of Skin Permeation[9]

Transdermal permeation of a drug involves the following steps:

1. Sorption by *Stratum corneum*.
2. Penetration of drug through viable epidermis.
3. Uptake of the drug by the capillary network in the dermal papillary layer

This permeation can be possible only if the drug possesses certain physiochemical properties.

The rate of permeation across the skin (dQ/dt) is given by:

$$\frac{dQ}{dt} = Ps(c_d - c_r) \text{----------(1)}$$

Where,

C_d – Concentration of the permeant in donor phase (*Stratum corneum*)

C_r – Concentration of the permeant in receptor phase (systemic circulation).

P_2 – Overall permeability co-efficient of the skin.

Permeability coefficient is given by the relationship:

$$Ps = \frac{K_2 D_2}{h_2} \text{=----------(2)}$$

Where,

K_2 - Partition co-efficient of penetrant.

D_2 - Apparent diffusivity of penetrant

h_1 - Overall thickness of the skin

Thus permeability coefficient (P_2) may be constant if K d and h terms are constant under a given set of conditions. A constant rate of drug permeation is achieved if $C_d >> C_2$

Then equation (1) becomes: $\frac{dQ}{dt} = P_2 C_d$----------(3)

The rate of skin permeation is constant provided the magnitude of C4 remains fairly constant throughout the course of skin permeation. For keeping C4 constant the drug should be released from the device at R_4 (release rate of drug) which is either constant or greater than R1 (the rate takes up the drug) i.e. $R_1 >> R_2$ Since $R_1 >> R_2$, the drug concentration on the skin surface C4 is maintained at a level equal to or greater than the equilibrium solubility of the drug in the SC, C, i.e. $C_d >> C_r$

Therefore a maximum rate of skin permeation is obtained and is given by the equation:

$(dQ/dt)_m = P_2 C_2$……..(4)

From the above equation it can be seen that the maximum rate of skin permeation depends upon the skin permeability coefficient P_2 and the equilibrium solubility in the SC, C_2. Thus skin permeation appears to be SC limited.

1.5.1. Enhancement of Transdermal Delivery:

Skin is an important site of drug application for both local and systemic effects. However in skin, the SC is the main barrier for drug penetration. Penetration enhancement technology is a challenging development that would increase the number of drugs available for transdermal administration. Significant advancement has been made, in the last two decades, in achieving a better control in the delivery through the skin. Research has been directed to find ways of delivering different types of drug molecule with the help of different enhancement techniques (Fig No.3)

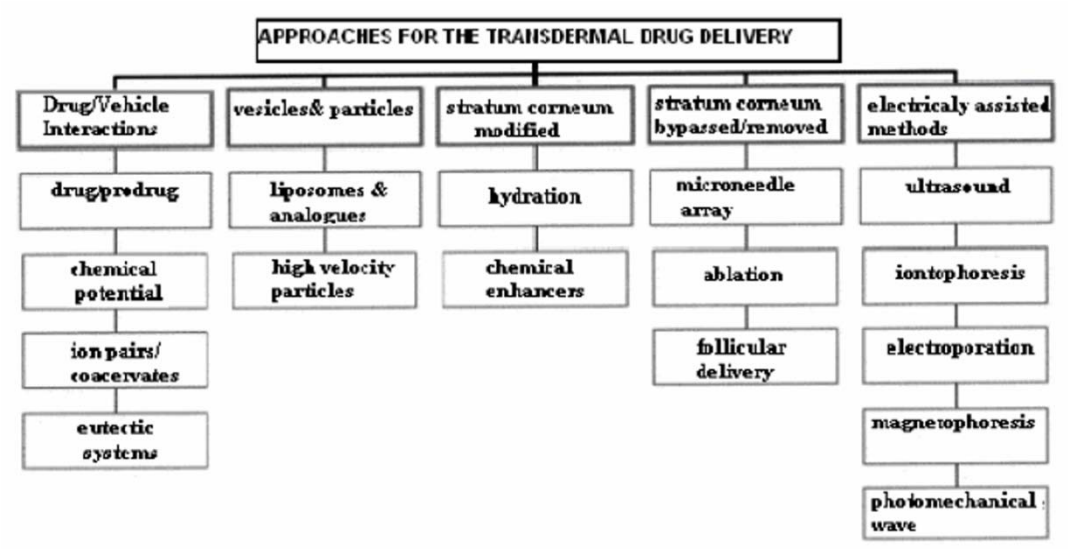


Fig No. 3: Various approaches to enhance drug delivery through skin.

A strategy to enhance the skin permeation in the present study is the use of permeation enhancers. One of the easiest approaches to enhance the permeation rate is the use of penetration enhancers. These are the substances added to pharmaceutical formulation in ordered to increase the membrane permeation or absorption rate of a co-administered drug.

1.5.2. Ideal characteristics of chemical penetration enhancers:

Ideally, penetration enhancers reversibly reduce the barrier resistance of the SC without damaging the viable cells. Some of the more desirable properties for penetration enhancers acting within the skin have been given as:

- ✓ They should be non-toxic, non-irritating and non-allergenic
- ✓ They would ideally work rapidly, and the activity and duration of effect should be both predictable and reproducible
- ✓ They should have no pharmacological activity within the body i.e. should not bind to receptor sites
- ✓ The penetration enhancers should work unidirectionally, i.e. should allow therapeutic agents into the body whilst preventing the loss of endogenous material from the body

- ✓ When removed from the skin, barrier properties should return both rapidly and fully
- ✓ The penetration enhancers should be appropriate for formulation into diverse topical preparations, thus should be compatible with both excipients and drugs
- ✓ They should be economicacally acceptable with an appropriate skin feel

1.5.3. Mechanism of Chemical Penetration Enhancement[10]

Penetration enhancers may act by one or more of three main mechanisms.

1. Disruption of the highly ordered structure of *Stratum corneum* lipid.
2. Interaction with intercellular protein.
3. Improved partition of the drug, co-enhancer or solvent into the Stratum coneum.

1.5.4. Different Classes of Permeation Enhancers are as follows:

1.5.4.1. Sulfoxides

Dimethylsulfoxide (DMSO) is an effective penetration enhancer that promotes permeation by reducing skin resistance to drug molecules or by promotion of drug partitioning from the dosage form. It has been postulated that DMSO denatures the intercellular structural proteins of the SC, or promotes lipid fluidity by disruption of the ordered structure of the lipid chains. Along with these, DMSO may alter the physical structure of the skin by elution of lipid, lipoprotein and nucleoprotein structures of the *Stratum corneum*.

1.5.4.2. Alcohols

Alcohols may influence transdermal penetration by a number of mechanisms. The alkyl chain length of the alkanols is an important parameter in the promotion of permeation enhancement. Lower molecular weight alkanols are thought to act as solvents, enhancing the solubility of drugs in the matrix of the *Stratum corneum*.

1.5.4.3. Polyols

The activity of propylene glycol is thought to result from salvation of a keratin within the SC; the occupation of proteinaceous hydrogen bonding sites reducing drug-tissue binding and thus promoting permeation.

1.5.4.4. Alkanes

Long chain alkanes (C_2-C_{16}) have been shown to enhance skin permeability by nondestructive alteration of the SC barrier.

1.5.4.5. Fatty acids

Selective perturbation of the intercellular lipid bilayers in the SC appears to be the major mode of enhancing activity of the fatty acids.

1.5.4.6. Esters

Esters such as ethyl acetate are relatively polar, hydrogen bonding compounds that may enhance permeation in a similar manner to the sulphoxides and formamides by penetrating into the SC and increasing the lipid fluidity by disruption of lipid packing.

1.5.4.7. Amines and amides:

- **Urea**

  Urea promotes transdermal permeation by facilitating hydration of the SC and by the formation of hydroplic diffusion channels within the barrier. Cyclical urea permeation enhancers are biodegradable, non-toxic molecules consisting of a polar parent moiety and a long chain alkyl ester group.

- **Dimethyl acetamide and dimethyl formamide**

  These compounds are less potent penetration enhancing chemical alternatives to DMSO. At low concentrations their activity as enhancers is a result of partitioning into the keratin regions. At higher concentrations they increase lipid fluidity by disruption of lipid packing as a result of salvation shell formation around the polar head groups of the lipids.

- **Pyrrolidones**

  Pyrrolidone and its derivatives are reported to interact with both keratin and with lipids in the skin. Azone is known to show significant accelerant effects at low concentrations for both hydrophilic and hydrophobic drugs and is one of the few enhancers that have been developed commercially. Differential scanning calorimetric studies have shown that azone affects lipid structures of the SC. In addition, azone is reported to decrease transition temperatures within lipid bilayers to induce formation of a liquid phase with a resultant increase in lipid fluidity.

1.5.4.8. Terpenes

Terpenes are found inessential oils, and are compounds comprising of only carbon, hydrogen and oxygen atoms, but which are not aromatic. Numerous terpenes have long been used as medicines as well as flavoring and fragrance agents. Terpenes are generally considered as less toxic and have less irritant effects compared to surfactants and other skin penetration enhacers, and some terpenes have been characterized as Generally Recognized As Safe (GRAS) by the US FDA. They have high percutaneous enhancement ability, reversible effect on the lipids of SC, minimal percutaneous irritancy at low concentrations (1-5%). Moreover, a variety of terpenes have been shown to increase percutaneos absorption of both hydrophilic and lipophilic drugs.[11] Both the mono-and sesquiterpenes are known to increase percutaneous absorption of compounds by increasing diffusivity of the drug and/or by disruption of the intercellular lipid barrier. A further mechanism of activity that has been postulated is that the terpenoids increase electrical conductivity of tissues thereby opening polar pathways within the *Stratum corneum*.

1.5.4.9. Surface active agents:

Surface active agents function primarily by adsorption at interfaces and thus interact with biological membranes contributing to the overall penetration enhancement of compounds. Cationic surfactants are more destructive to skin tissues causing a greater increase in flux than anionic surfactants. The latter, in turn, produce greater increases in flux than non-ionic surfactants.

Anionic surfactants may function by alteration of the barrier function of the SC as a result of removal of water soluble agents that act as plasticizers. Sodium lauryl sulphate has been implicated in reversible lipid modification with resultant disorganization of the SC and enhanced permeation. In addition, non-ionic surfactants are purported to be able to emulsify sebum, consequently altering partitioning potential of drugs in favour of enhanced permeation. The permeation enhancement generated by these compounds may be dependent on the ability of drug to partition between the free and bound or micellar form of the enhancer.

1.5.3.10. Cyclodextrins

Cyclodextrins are bio-compatible substances that can form inclusion complexes with lipophilic drugs with a resultant increase in their solubility, particularly in aqueous solutions. However, cyclodextrins alone were determined be to less effective as penetration enhancers than when combined with fatty acids and propylene glycol.

1.6. Basic Components of Transdermal Drug Delivery Systems[11]

The components of transdermal devices include:

1. Backing layer
2. Drug containing reservoir
    a. Polymer matrix
    b. Drug
    c. Permeation enhancers
    d. Plasticizers
3. The release control layer
4. The adhesive
5. The peel strip.

1. Backing layer

Backing layer must be impermeable to drugs and enhancers if used and as a result it is usually unpermeable to water vapor that is occlusive.

The most commonly used backing materials are alupoly, polyester, polyethylene co-extruded films or metalized polyester laminated with polyethylene. The film can be either clear flesh colored or metalized. Backing membranes are flexible and provide a good bond to the drug reservoir prevent drug from leaving the dosage form through the top and accept printing. It protects the product during use on the skin.

2. Drug containing reservoir:

A) Polymer Matrix:

The Polymer controls the release of the drug from the device. The development of transdermal systems requires judicious selection of a polymeric material or a series of polymers whose diffusive characteristic will be such that a desirable permeation rate of a specific drug or other bio-active agent can be obtained.

The polymer should meet the following requirements:

i) Molecular weight, glass transition temperature and chemical functionality of polymer must allow proper diffusion and release of the specific drug.

ii) The polymer should not chemically react with the drug.

iii) The polymer and its degradation products must be non-toxic.

iv) The polymer should not decompose on storage or use of the device.

v) The polymer should be inexpensive.

vi) The polymer must be easy to manufacture and it should yield itself into the desired product and should allow incorporation of large quantities of active component without deterioration of its mechanical properties.

Polymers that can be used in the transdermal formulations are:

a) Natural Polymers:

Cellulose derivatives, Zein, Gelatin, Shellac, Waxes, Proteins, Gums and their derivatives, Natural rubber, Starch etc.

b) Synthetic Elastomers:

Polybutadieine, Hydrin rubber, Polysiloxane, Silicone rubber, Nitrile, Acrylonitrile, Butyl rubber, Styrenebutadieine rubber, Neoprene etc.

c) Synthetic Polymers:

Polyvinyl alcohol, Polyvinyl chloride, Polyethylene, Polypropylene, Polyacrylate, Polyamide, Polyurea, Polyvinylpyrrolidone, Poly methylmethacrylate, etc.,

B) Drug:

For successfully developing a TDDS, the drug should be chosen with great care. The following are some of the desirable properties of a drug for transdermal delivery.

Physicochemical properties:

1. The drug should have a molecular weight less than approximately 1000 daltons.
2. The drug should have affinity for both lipophilic and hydrophilic phases.
3. The drug should have a low melting point.

Biological properties:

1. The drug should be potent with a daily dose of the order of a few mg/day.
2. The halflife (tn) of the drug should be short.
3. The drug must not induce a cutaneous irritant or allergic response.
4. Drugs which degrade in the g.i.t or/are inactivated by hepatic first pass effect are suitable candidates for transdermal delivery.
5. Tolerance to the drug must not develop under the near zero order release profile of transdermal delivery.
6. Drugs which have to administered for along period of time or which cause adverse effects to non target tissues can also be formulated for transdermal delivery.

C) Permeation Enhancers:

Discussed in detail in section 1.5.

D) Plasticizers:

These are used to prevent the films from becoming brittle.[12] An ideal plasticizer should possess the following properties.

1. Should not show any pharmacological action of its own.
2. Should be chemically and physically stable.
3. Should be compatible with the drug and the formulated components.
4. Should be colourless, colorless and tasteless.
5. Should be non-toxic, non-allergenic & non-irritant.

3.Release control layers:

The rate controlling membrane can be either a micro-porous or a non-porous polymeric membrane with defined drug permeability. The membrane can be constituted with any of the polymers discussed earlier.

4. Adhesives:

The fastening of all transdermal devices to the skin has so far been done by using a pressure sensitive adhesive which can be positioned on the face of the device or in the back of the device and extending peripherally. Both adhesive systems should fulfill the following criteria.

(i) Should adhere to the skin aggressively, should be easily removed.
(ii) Should not leave an unwashable residue on the skin.
(iii) Should not irritate or sensitize the skin.

The face adhesive system should also fulfill the following criteria.

i) Physical and chemical compatibility with the drug, excipients and enhancers of the device of which it is a part.
ii) Permeation of drug should not be affected.
iii) The delivery of simple or blended permeation enhancers should not be affected.

5. Peel Strip:

The peel strip prevents loss of drug that has migrated into adhesive layer during storage and also protects the finished device against contamination. Polyester foils and metalized laminates are the choices.

6. Packet:

Packet guards the patches against drug loss and contamination on storage. The patches are individually packed in heat sealed foil pouches.

1.7 . Approaches used in the Development of TDDS [12]

Several techniques have been successfully developed to provide a mechanism of rate control over the release and transdermal permeation of drugs. These are three types of devices currently available in the market. To be precise, there are two concepts in the design namely, the reservoir type and the matrix type.

1.7.1 Different technologies employed in the development of TDDS:

I. Polymer Membrane Permeation controlled TDDS:

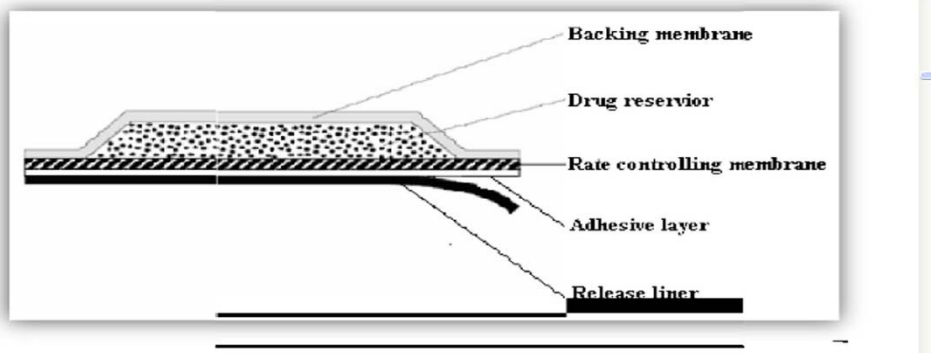


Fig No. 4: Cross section view of Polymer Membrane Permeation-Controlled TDDS

In this system the drug reservoir is sandwiched between a drug impermeable backing laminate and a rate controlling polymeric membrane. The drug molecules are permitted to release only through the rate controlling polymeric membrane.

In the drug reservoir compartment the drug solids are dispersed homogeneously in a solid polymer matrix (e.g., poly isobutylene), suspended in an unleachable, viscous liquid medium (e.g., silicone fluid) to form a pase like suspension, or dissolved in a releasable solvent (e.g.,alkyl alcohol) to form a clear drug solution. On the external surface of the polymeric membrane a thin layer of drug compatible, hypoallergenic pressure sensitive adhesive polymer (e.g., silicone adhesive) may be applied to provide intimate contact of the TDDS with the skin surface. The intrinsic rate of drug release from this type of TDDS is defined by:

$$\frac{dQ}{dt} = \frac{K_{m/r} K_{l/r} D_2 D_m}{K_{m/r} D_m h_2 + K_{2/m} D_2 h_m} C_r \text{ ----------} \rightarrow (5)$$

Where: $\mathbf{C_R}$ – Drug concentration in the reservoir compartment.

$\mathbf{K_{m/r}}$**&** $\mathbf{K_{a/m}}$ -- Are the partition coefficients for the interfacial partitioning of drug from the reservoir to the membrane and from the membrane to the adhesive.

$\mathbf{D_m}$**&** $\mathbf{D_2}$ --- Are the diffusion coefficients in the rate-controlling membrane and in the adhesive layer.

$\mathbf{h_m}$**&** $\mathbf{h_a}$ -- are the thickness of rate-controlling membrane and adhesive layer.

II. Polymer matrix Diffusion-Controlled TDDS:

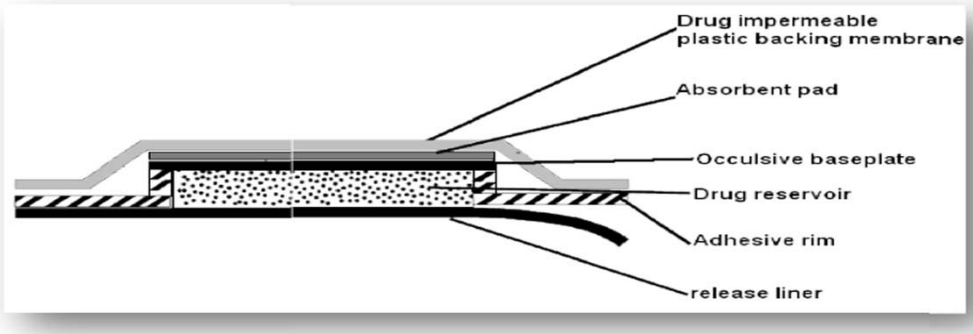


Fig No. 5: Cross section view of Polymer Matrix Diffusion-Controlled TDDS.

In this approach the drug reservoir is formed by homogeneously dispersing the drug solid in a hydrophilic or lipophilic polymer matrix and the medicated polymer

formed is then molded into medicated disks with a defined surface area and controlled thickness. This drug reservoir containing polymer disk is then mounted onto an occlusive baseplate in a compartment, fabricated from a drug impermeable plastic backing. In this system the adhesive polymer is applied along the circumference of the patch to form a strip of adhesive rim surrounding the medicated disk. The rate of drug release from this polymer matrix drug dispersion type TDDS is given by:

$$\frac{dQ}{dt} = \left[\frac{L_d C_p D_p}{2t}\right]^{1/2} \text{----------} \rightarrow (6)$$

Where:

L_d -- Drug loading dose initially dispersed in the polymer matrix.

C_p& D_p – Solubility and diffusivity of the drug in the polymer matrix.

t – Time.

III.Drug Reservoir Gradient-Controlled TDDS:

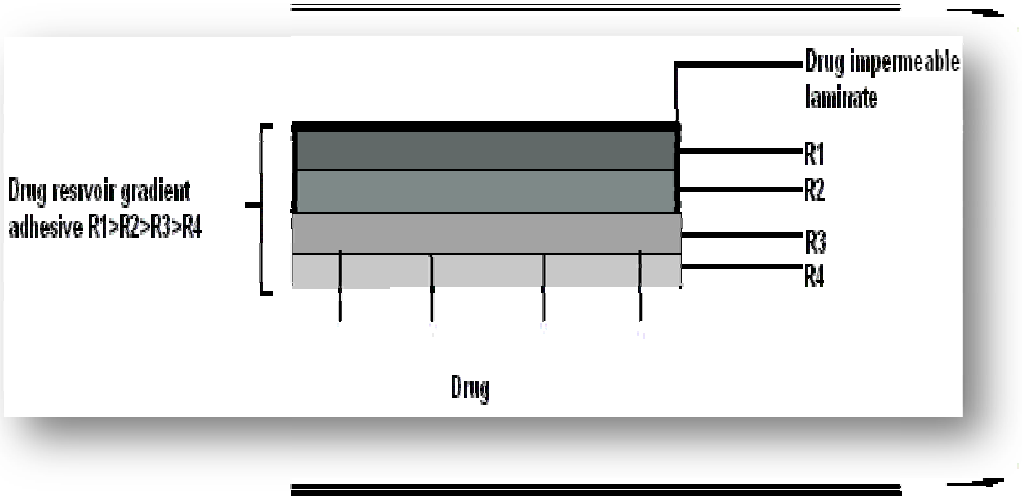


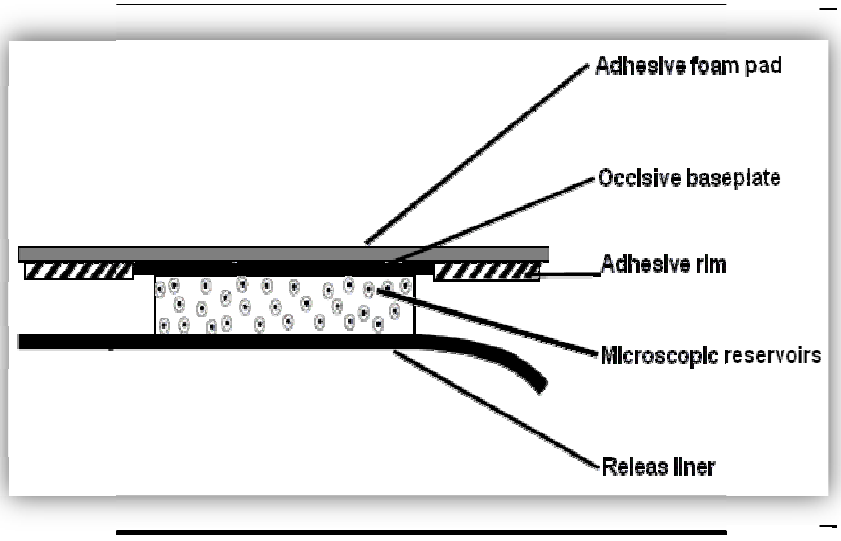
Fig No.6: Cross section view of Drug Reservoir Gradient-Controlled TDDS

The polymer matrix drug dispersion type TDDS can be modified to have the drug loading level varied in an incremental manner, forming a gradient of drug reservoir along the diffusional path across the multilaminate adhesive layers. The rate of drug release from this type of drug reservoir gradient controlled TDDS can be expressed by:

$$\frac{dQ}{dt} = \frac{K_{a/r} D_2}{h_2(t)} L_d(h_2) \text{----------} \rightarrow (7)$$

In this system the thickness of diffusional path through which drug molecules diffuse increases with time, i.e., $\mathbf{h_a(t)}$. To compensate for this time dependent increase in diffusional path as a result of drug depletion due to release, the drug loading level in the multilaminate adhesive layers is also designed to increase proportionally, i.e., $\mathbf{L_d(h_a)}$. This, in theory should yield a more constant drug release profile.

IV.Microreservoir Dissolution Controlled TDDS



FigNo.7: Cross section view of Microreservoir Dissolution Controlled TDDS

This type of drug system can be considered a hybrid of the reservoir and matrix dispersion-type drug delivery systems.

In this approach the drug reservoir is formed by first suspending the drug solids in an aqueous solution of a water-miscible drug solubilizer, e.g., polyethylene glycol and then homogeneously dispersing the drug suspension with controlled aqueous

solubility in a lipophilic polymer by high shear mechanical force to form thousands of unleachable microscopic drug reservoirs. This thermodynamically unstable dispersion is quickly stabilized by immediately cross linking the polymer chains in-situ, which produces a medicated polymer disk with a constant surface area and a fixed thickness. The rate of drug release from a microreservoir drug delivery system is defined by:

$$\frac{dQ}{dt} = \frac{D_p D_r A K_2}{D_p h_d + D_2 h_2 A K_2}\left[BS_p - \frac{D_1 S_1 (1-B)}{h_1}\left(\frac{1}{k_m} + \frac{1}{k_m}\right)\right] - - - - - - - - - - - - - > (7)$$

Where:

A = a/b, a is the ratio of the drug concentration in the bulk of elution solution over the drug solubility in the same medium, and b is the ratio of the drug concentration at the outer edge of the polymer coating membrane over the drug solubility in the same polymer composition.

B = is the ratio of the drug concentration at the inner edge of the interfacial barrier over the drug solubility in the polymer matrix.

K_l, K_m & K_p = are the partition co-efficients for the interfacial partitioning of drug from the liquid compartment to the polymer matrix, from the polymer matrix to the polymer coating membrane, and from the polymer coating membrane to the elution solution (or skin).

D_l, D_p & D_s – are the drug diffusivities in the liquid compartment, polymer coating membrane, and elution solution (or skin), respectively.

S_l & S_p – are the solubilities of the drug in the liquid compartment and in the polymer matrix, respectively and **h_l, h_p & h_d** – are the thickness of the liquid layer surrounding the drug particles, the polymer coating membrane around the polymer matrix, and the hydrodynamic diffusion layer surrounding the polymer coating membrane, respectively.

1.8 Use of Transdermal Patch

Fig No.8: Use of Transdermal Patch

It is important to use a different application site every day to avoid skin irritationSuggested rotation is :

Day 1 - Upper right arm

Day 2 - Upper right chest

Day 3 - Upper left chest

Day 4 - Upper left arm,

Then repeat from day 1.

1.8.1. Transdermal Patches Available in the Market[13]

Nowadays, the transdermal route has become one of the most successful and innovative focus for research in drug delivery with around 40% of the drug candidate being under clinical evaluation related to transdermal or dermal systems.

The market for transdermal product has been in a significant upward trend and this is likely to continue to deliver real therapeutic benefit to patients around the world. More than 35 TDDS products have now been approved for sale in the US, and approximately 16 active ingredients have been approved for use globally.

Statistics reveal a market of $ 12.7 billion in the year 2005 that is expected to increase to $ 21.5 billion in the year 2010 and $ 31.5 billion in the year 2015. The pie diagram shows the global transdermal product sales.

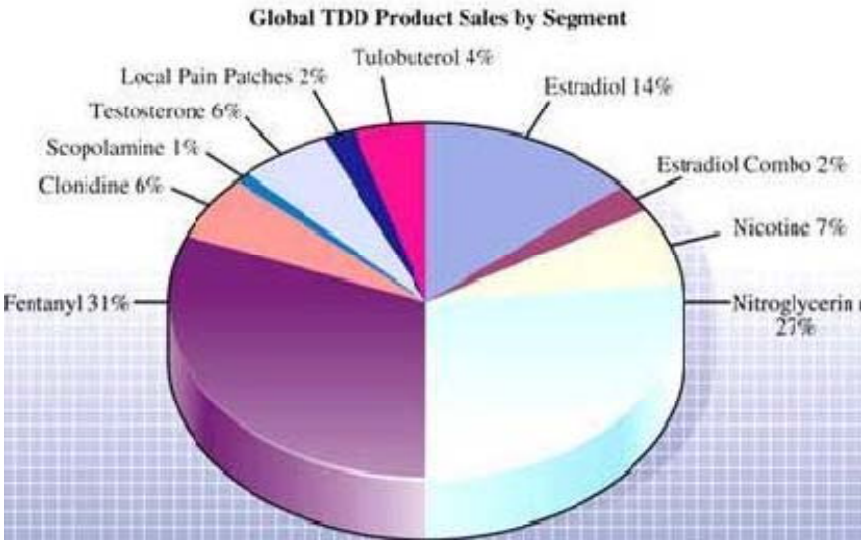


Fig No. 9: Pie diagram of popularly marketed Transdermal patch

Table No .1: List of Transdermal Patches available in The Market

Brand Name	Drug	Manufacturer	Indications
Nicotinell®	Nicotine	Novartis	Pharmacological smoking cessation
Matrifen®	Fentanyl	Nycomed	Pain relief patch
Neupro®	Rigotine	UCB and Schwarx pharma	Early-state idiopathic parkinson's disease
Alora	Estradiol	Thera Tech/ Procter and gamble	Postmenstrual syndrome
Nitrodisc	Nitroglycerin	Robert's Pharmaceuticals	Angina pectoris
Nuvelle TS	Estrogen/ Progesterone	Ethical Holdings/ Schering	Hormone replacement therapy
Testoderm TTS®	Testosterone	Alza	Hypogonadism in males
Oxytrol®	Oxybutynin	Watson Pharma	Overactive bladder
Catapres TTS®	Clonidine	Alza/Boehinger Ingelheim	Hypertension
Minitran	Nitroglycerin	3M Pharmaceuticals	Angina pectoris
Duragesic	Fentanyl	Alza/Jannssen Pharmaceutical	Moderate/ severe pain
Emsam	Selegiline	Bristol-Myers Squibb	Major depressive disorder
Neupro	Rotigotine	Schwarz Pharma	Parkinson's Disease

Exelon	Rivastigmine	Novartis	Dementia
Daytrana	Methylphenidate	Shire	Attention deficit hyperactivity disorder
Synera	Lidocaine/ tetracaine	Endo Pharmaceuticals	Local dermal Analgesia
Ionsys	Fentanyl HCI (iontophoresis)	Alza	Acute postoperative pain
Scnoprep	Lidocaine (ultrasound)	Echo Therapeutics	Local dermal Anesthesia
Oxytrol	Oxybutynin	Watson Pharma	Overactive Bladder
Climara Pro	Estradiol	Bayer Healthcare Pharmaceuticals	Menopausal symptoms
Ortho Evra	Ethinyl estradiol/ norelgestromin	Ortho-Mc Neil Pharmaceutical	Contraception
Nicoderm,Habitrol,Prostep	Nicotine	Glaxo Smith Kline, Novartis Consumer Health, Elan	Smoking cessation
Testoderm	Testosterone	Alza	Testosterone Deficiency
Iontocaine	Lidocaine/ epinephrine (iontophoresis)	Iomed	Local dermal Analgesia
Combipatch	Estradiol/ norethidrone	Novartis	Menopausal symptoms

1.9 . Therapeutic Applications of TDDS[13]

- Hisetal, used in treatment of multiple sclerosis can be formulated in TDDS using oleic acid as permeation enhancer to achieve sufficient drug delivery.
- Diclofenac sodium, Celecoxib used as NSAID's, formulated in TDDS can overcome gastric lesions associated with oral dose.
- Drugs used for long term dosing in chronic diseases like captopril, verapamil, terbutaline sulphate, pinacidil, propranolol which have short biological half life, considerable first pass metabolism can be formulated as TDDS to achieve prolonged steady state plasma concentration.
- Gel formulation with lipid disperse system of betahistine has potential for development of an efficient controlled release transdermal system.
- Enhancer and co-solvent can synergictically enhance the delivery of peptides like thyrotropin releasing hormone across human skin.
- Prazosin HCL in membrane controlled TDDS can deliver drug enough to maintain minimum effective concentration and can avoid hypotension associated with high initial oral dosing.
- TDDS of indomethacin in polyvinylpyrolidone polymer (acting as anti nucleating agent) can provide better anti-inflammatory activity and lower ulcer indices compared to oral administration.
- Diclofenac sodium, existing in anionic form at skin pH can be formulated as ion- pairs with oppositely charged enhancers to enhance transdermal delivery compared to non-ion paired forms.
- Iontophoresis can increase permeation rate of hydrophilic atenolol to a greater extent than permeation enhancer and overcome incomplete absorption on GIT.
- Nimesulide in sodium alginate transdermal gel can provide better analgesic and anti-inflammatory activity and avoid adverse effects associated with long term treatment with high oral dose.
- Terbutaline sulphate, being dia magnetic can be incorporated in magnetic TDDS to experience driving force to escape from applied magnetic field and enhance diffusion across the skin.

- Bupropion HCl, an antidepressant drug can be converted to free base to increase lipophilicity and transdermal delivery and avoid release of fatal metabolites associated with oral dosing.
- Zidovudine, an anti-HIV drug, formulated in TDDS and overcome toxic effects associated with frequent higher oral dose.
- Levonorgestrel, a potent contraceptive agent, formulated as transdermal protransferosome gel can provide enhanced, prolonged and controlled delivery and overcome GI disturbances, weight gain, irregular bleeding, headache etc. associated with oral dosing.
- Polymerized rosin can be used ti design matrix type TDDS of Diltiazem HCl to prolong drugrelease and avoid variable and extensive first pass metabolism on oral dose.
- Ester prodrug of ketorolac can provide enhanced permeation whereas nanostructured lipid carrier can act as controlled release system and avoid gastric ulceration and renal failure associated with frequent long term oral dosing.

1.10. Limitations of Transdermal Delivery System

- Higher molecular weight candidates (>500Daltons) fail to penetrate the *Stratum corneum*.
- Drugs with very low or high partition coefficient fail to reach systemic circulation.
- High melting drugs are not suitable due to their low solubility both in water and fat.
- Possibility of local irritation at the site of patch application.
- A lag time associated with the delivery of the drug across the skin, resulting in a delay in onset of action.
- Variation of absorption rate based on site of application.
- Presence of skin diseases.
- Variation in adhesive effectiveness in different individuals.

1.11. Evaluation of Transdermal Patches [14]

Development of controlled release transdermal dosage form is a complex process involving extensive research. Transdermal patches have been developed to improve clinical efficacy of the drug and to enhance patient compliance by delivering smaller amount of drug at a predetermined rate. This makes evaluation studies even more important in order to ensure their desired performance and reproducibility under the specified environmental conditions.

These studies are predictive of transdermal dosage forms and can be classified into following types.

I. Physicochemical evaluation
II. *In-vitro* evaluation
III. *Ex-vivo* evaluation.

Upon the success of physicochemical and *in-vitro* studies, *in-vivo* evaluations may be conducted.

I. Physicochemical Evaluation:

1. **Thickness:** The thickness of transdermal film is determined by screw gauge at different points of the film.[15]
2. **Unformity of weight:** Weight variation is studied by individually weighing 10 randomly selected patcvhes and calculating the average weight. The individual weight should not deviate significantly from the average weight.[16]
3. **Drug content determination:** An accurately weighed portion of film (about 100 mg) is dissolved in 100 ml. of suitable solvent in which drug is soluble and then the solution is shaken continuously for 24 h in shaker incubator. Then the whole solution is sonicated. After sonication and subsequent filtration, drug in solution is estimated spectrophotometrically by appropriate dilution.[17]
4. **Content uniformity test:** 10 patches are selected and content is determined for individual patches. If 9 out of 10 patches have content between 85% to 115% of the specified value and one has content not less than 75% to 125% of the specified value, then transdermal patches pass the test of content uniformity.

But if 3 patches have content in the range of 75% to 125%, then additional 20 patches are tested for drug content. If these 20 patches have range from 85% to 115% then the transdermal patches pass the test.

5. **Moisture content:** The prepared films are weighed individually and kept in a desiccators containing calcium chloride at room temperature for 24 h. The films are weighed again after a specified interval until they show a constant weight. Percent moisture content is calculated using following formula.[18]

   % Moisture content = $\frac{\text{Initial weight – Final weight}}{\text{Final weight}}$ X 100

6. **Moisture Uptake:** Weighed films are kept in a desiccator at room temperature for 24 h. These are then taken out and exposed to 84% relative humidity using saturated solution of potassium chloride in a desiccator until a constant weight is achieved. Percent moisture uptake is calculated as given below.[18]

   % moisture uptake = $\frac{\text{Final weight – Initial weight}}{\text{Initial weight}}$ X 100

7. **Folding Endurance:** Evaluation of folding endurance involves determining the folding capacity of the films subjected to frequent extreme conditions of folding. Folding endurance is determined by repeatedly folding the film at the same place until it break; the number of times the films could be folded at the same place without breaking is folding endurance value.[18]
8. **Tensile Strength:** To determine tensile strength, polymeric films are sandwiched separately by corked linear iron plates. One end of the films is kept fixed with the help of an iron screen and other end is connected to a freely movable thread over a pulley. The weights are added gradually to the pan attached with the hanging end of the thread. A pointer on the thread is used to measure the elongation of the film. The weight just sufficient to break the film is noted. The tensile strength can be calculated using the following equation.[20]

Tensile strength = F/a.b(1=L/1)

F is the force required to break; a is width of film; b is thickness of film; L is length of film; 1 is elongation of film at break point.

II. The *in-vitro* evaluation [5,6]

The objective of in-vitro research is often to find correlation between laboratory results and the transdermal absorption experienced by living subjects, so that, *in-vivo* experimentation may be curtailed.

The factors to be considered while selecting an *in-vitro* system include:

1. The rate limiting process: drug solubization or diffusion in the vehicle, partitioning from the vehicle, diffusion through the test membrane or partitioning and removal by the receptor phase.
2. The intrinsic diffusivity of the permanent and apparent diffusivity.
3. The predominating route of diffusion during the experiment and the relative extents of drug binding the metyabolism, occurring in the membrane, delivery and receptor phases.
4. The intrinsic barrier potential of the membrane and the effects that vehicle components may have on its retardative properties. Hydration of the membrane and the presence of penetration enhancers may be important here. Inter-specimen variability between membranes of the same type may markedly influence experimental results.

   The various types of cells lused for carrying out in-vitro release studies are

   1. Chien and Vilia cell
   2. Franz diffusion cell
   3. Keshary-chien diffusion cell
   4. USP XXIII’s ge berak drug release standards for TDDS.

Usually Diffusion Cell Design contains two parts donor and received compartment:

All materials should be assessed for their ability to absorb or adsorb the test penetrant.

1. **Donor Compartmen**t is designed in such a way to achieve
    - ❖ Easy access to deliver the penetrants to the skin.
    - ❖ (37 ± 1^{o})c of temperature.
    - ❖ Control of evaporation for volatile vehicles and penetrants.

    Membrane used in study should have following characteristics:

    - ❖ For the study of penetration kinetics, only porcine epithelium should be used.
    - ❖ For vehicle/device release studies, other barriers may be used.
    - ❖ The skin sample should ideally contain only SC.
    - ❖ A molecule of known penetration kinetics should be used prior to the test molecule, to assess barrier function.
    - ❖ Wherever applicable, metabolic viability of epidermis must be assessed.
2. **Receptor Compartment**
    - ❖ Either, flow-through or static.
    - ❖ (37 ± 1^{o})c of temperature
    - ❖ Sufficient volume to maintain infinite sink conditions.
    - ❖ Stirred without obvious formation of boundary layers.
3. **Receptor Fluid**
    - ❖ Should not compromise barrier function.
    - ❖ Should be of favorable partitioning characteristics to receive the penetrant.
    - ❖ Capable of maintaining epidermal viability wherever necessary.
    - ❖ Must be contained once collected.

A vast majority of *in-vitro* experiments are conducted on animal skin i.e., hairless mouse, guinea pig and rabbit too. Although there exists a number of similarities there is as yet no animal skin that completely mimics the penetration characteristics of human skin.

III. *Ex-vivo* evaluation [5,6]

Although most relevant data pertaining to TDDS are obtained in human subjects, this desirable approach is not always possible, since considerable time & resources are required to conduct a safe & meaningful percutaneous absorption studying man. Consequently, one must be preferred to use an *in-vivo* animal model.

Although the range of species employed in previous work is very broad, there is no general agreement as to the best or most predictive model for skin penetration work. The various animal models those have been utilized for the in-vivo studies are mouse, rat, guinea pig, rhesus monkey, rabbit, dog etc.

1.12. SCABIES[21]

Scabies is a skin disease caused by a mite called *Sarcoptesscabiei*, which is a parasite that burrows into, resides and reproduces in human skin. It affects people of all ages. However, people with weakened immunity or the elderly are more susceptible to scabies. Hence, outbreaks of scabies have been reported in hospitals, child-care facilities, hostels and elderly homes.

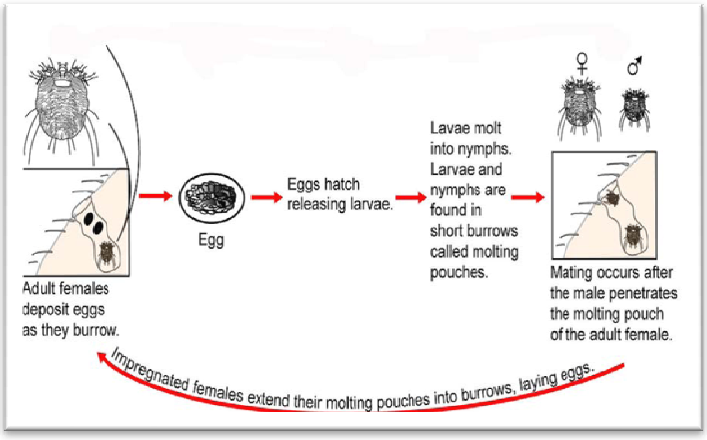


Fig No. 10: Life Cycle of Scabies mite

1.12.1 . Clinical features

Scabies manifests in two main ways: People infected with classical scabies will present with intense itchiness which is more severe at night or after a bath. The common affected areas are finger webs and skin folds of wrists, elbows, armpits, nipples, lower abdomen, external genitalia, buttocks and groins. Rashes, thread-like lesions or vesicles may be seen on the skin. The face and scalp are usually spared, except in infants, young children and immune-compromised persons.

A rare but severe type of scabies known as Norwegian or crusted scabies can occur in institutionalized people, particularly in those who are weak or mentally disabled. The skin lesions appear as marked scales and crusts. The nails may thicken, with debris in the nail bed. Face and scalp can also be affected. This type of scabies is highly contagious because an infected person may harbour thousands of mites, compared with 10-15 mites present in classical scabies.

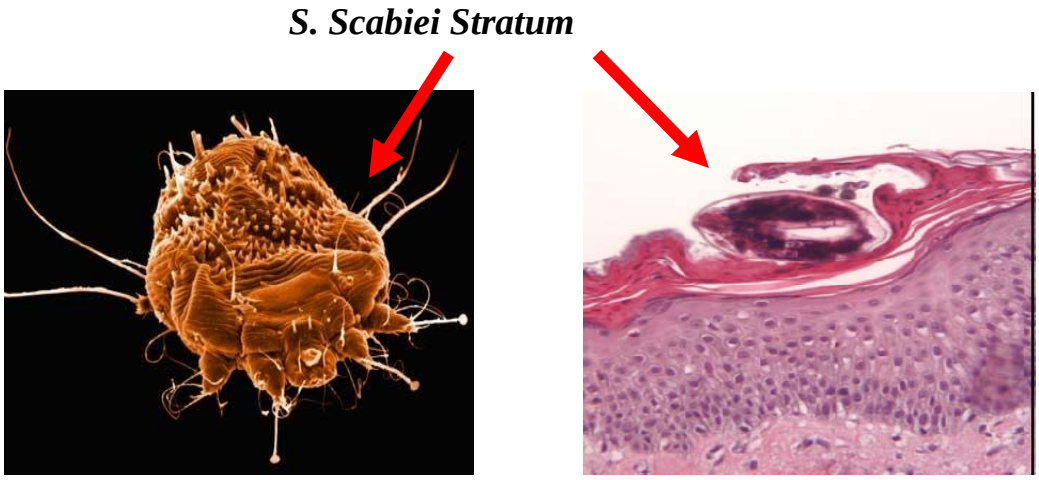
1.12.2. Path physiologys of Scabies[22]

- **Mode of transmission**

  Scabies usually spreads through direct skin contact with the infested people. Their clothing and bedding may also carry the mites/eggs and transmit the disease. Transmission within household and institutional setting is common.

- **Incubation period**

  Incubation period is about 2-6 weeks for people without previous exposure to the disease. For people who have previously been infested with scabies, symptoms develop earlier, usually within 1-4 days after re-exposure.



A **B**

Fig No.11:A. Adult female *S. scabiae,* ventral (stomach) view, with internal egg

B. Adult scabies mite (cross-section) in outer layer of skin (*Stratum corneum*)

- **Management**

- If you suspect scabies, seek medical advice immediately
- Apply topical medicated lotions and take drugs according to the doctor's advice to kill the mites and control itchiness respectively
- Family member(s) and sexual partner(s) of the infested persons should also seek medical advice and receive treatment if necessary
- When caring for and coming into contact with the infested persons, were appropriate personal protective gear such as gloves and apron
- Wash hands thoroughly before and after contact with the infested person
- Wash the clothing, towels and bed linen of the infested persons separately. These items should be washed in hot water at 60°C or above, for not less than 10 minutes so as to kill the mite and its eggs. Clean equipment for shared use with detergent before using on other persons. Place non-washable items in a plastic bag and seal up for at least 14 days before reuse

1.12.3. Prevention

- Keep good personal hygiene, wash hands and body frequently
- Regularly change into clean clothing, towels and bed linen
- Avoid sharing clothing and personal items with others
- Perform skin inspection for institutionalized residents regularly for early identification of infestation

2. LITERATURE SURVEY

2.1. Literature Review of TDDS :

- **Santhosh K *et al*., 2013** Formulated and Evaluated Ketoprofen Transdermal Patches.They concluded that the 4:1 ratio formulation using ethyl cellulose and PVP showed best drug release (97.87%) in 24 hrs[23]

- **Vennadeepthi *et al.*, 2013** Formulated and Evaluated Transdermal Patch of Anti hypertensive drug(Atenolol). They concluded that the F4 formulation prepared by using HPMC: Eudragit RL 100(4:3 ratio) showed controlled release up to 24 hrs[24]

- **SwethaVennapossa *et al.*, 2013** Formulated and Evaluated Matrix type Tran sdermal Patch containing Rivastigmine Tartarate. They concluded that the i*n-vitro* drug release studies revealed that the formulation F3 provided sustained release due to increase in amount of EC in the formulation. More or less similar trend was studied in case of F2 and F1 formulations. When the average rate constant of these 3 formulations were compared it was found that F3(PVP:EC-1:5) had the slowest release rate of all the formulations studied. Therefore based on the above observations it can be reasonably concluded that PVP-EC (1:5) polymers are better over PVP-EC (1:1&1:3) for the development of TDDS of Rivastigmine Tartarate[25]

- **Kansagra Hemanh *et al.*, 2012** Formulated and Evaluated Transdermal Patch of Sertaconazole nitrate. The permeation studies illustrated that the ratio of polyvinyl pyrrolidone and ethyl cellulose 1:5 showed good controlled release. Higuchi and Korsmeyer-Peppas models were used for optimizing the formulation[26]

- **Kunal N Patel *et al.*, 2012** Formulated and Evaluated Transdermal Patches of Diclofenacacid. The prepared patches showed good uniformity with regard to drug content and drug release. Formulation F3 has achieved the targets of study such as extended release, avoidance of first pass metabolism, reduced frequency of administration and thus may improve the patient compliance[27]

- **G. Parthasarathy *et al.*, 2011** Designed, Formulated and Evaluated Transdermal drug Patches of Naproxen with various polymers. The study showed that the ethylcellulose and Hydroxy propyl methyl cellulose along with the plasticizer dibutylphthalate 10% w/v of polymer weight was suitable for good flexibility and elasticity[28]

- **Sarswathi R *et al.*, 2010** Formulated and Evaluated Transdermal Patches of Curcumin.The *in - vitro* permeation studies of patches was carried out using 0.5% Sodium Lauryl Sulphatesolution in the receptor compartment with their different patches, F1, F2 and F3 using HPMC and EC bring the satisfactory release of curcumin. The cumulative percentage drug release of patch F1-82.20%, F2-74.06% and F3-68.27%. The release kinetics was evaluated making by use of Zero order, first order, Higuchi's diffusion and Korsemeyer-Peppas equation. The drug release through transdermal patches of curcumin followed Zero order kinetics[29]

- **Rakesh .P. Patel*et al.*, 2009** Formulated and Evaluated Transdermal Patch of Aceclofenac. The study indicated that as the drug permeation increased with increased concentration of penetration enhancer[30]

2.2. Literature Review of Scabies:

- **Johnston G *et al.*,(2011)** reviewed the diagnosis and treatment of Scabies [19]

- **Chosidow O, (2006)** reviewed the clinical practices of Scabies[20]

- **Stanley scheindlin *et al.*, (2004)** discussed transdermal drug delivery: past, present and future scope in molecular interventions[21, 22]

2.3. Literature Review of *Acalypha indica Linn.*

- **Jagatheeswari *et al.*, (2013)** carried out a review of its traditional uses and pharmacological properties. Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine. This article aims to provide a comprehensive review on the and pharmacological aspects of *Acalypha indica* Linn.[31]

- **Siva *et al.*, (2009)** carried out the study on four different extracts of whole plant of *Acalypha indica Linn.* . They tested for post-coital anti fertility activity in female albino rats. Of these, only pet.ether and ethanol extracts were found most significant activity. But activity is reversible on withdrawal of extract[32]

- **Siddique *et al.*, (2008)** carried out the aqueous extract of *Acalypha indica Linn.* were studied for Neuro - protection and neuro-therapy effect *ex-vivo* on musculus gastro frog studies were done on two groups of frog, the parameters measured in this study are electrical activities such as amount and duration (in second) of repolarization, depolarization, resting potential, and height of spike after electrical stimulation. Extract showed both activities[33]

- **M.Karthikeyan** *et al., (*2008) Extracts of leaves of *Acalypha indica* Linn. was evaluated for antibacterial activity against both gram positive and gram-negative bacteria. All extracts shown activity against various strains of gram positive and in gram negative only on *Pseudomonas aeruginosa*[34]

- **Dibinlal** *et al., (*2008) Fresh juice of *Acalipha indica* Linn. leaf were evaluated for anti-inflammatory activity on four groups of albino rats which are pretreated orally with control, standard, (indomethacin), in combination of both *Acalypha indica* Linn. & Indomethacin, *Acalypha indica* Linn. juice was effective in inhibition of paw volume and oedema[34]

- **Kannan** *et al.,* (2008) carried out the different leaf extract of *Acalypha indica* Linn. tested for larvicidal against *Anapheles stephensi*. the ability of ovicidal activity was observed, the percent was versely proporitional to concentration of extract and directly proportional to the eggs. Result of extracts having promising activity[35]

- **Rajeshwari** *et al., (*2007) synthesized nanoparticles using leaf extract of *Acalypha indica* Linn. The antibacterial activity of synthesized silver nanoparticles showed effective inhibitory activity *against* water borne pathogens Viz., *Escherichia coli* and *Vibrio cholerae*[36]

3.AIM AND OBJECTIVE

3.1. Aim

To prepare transdermal patch of Methanolic Extract of *Acalypha indica* Linn. using different polymers for the treatment of scabies.

3.2. Objective

- To formulate the herbal extract into a novel dosage form
- To prepare Transdermal patch of Methanolic Extract of *Acalypha indica* Linn.using different polymers and to evaluate its effective release to treat scabies
- To provide a direct entry of drug into the systemic circulation to give immediate relief and to give sustained action

The Transdermal route is easily accessible for self-medication andsafe, hence it is well accepted by patients. Since the device can be easily adhered on to the skin and removed from the site of application.

4. PLAN OF THE WORK

- Literature Survey
- Selection of Herb
- Collection of leaves of *Acalypha indica* Linn.
- Authentication
- Extraction (Soxylation method)
- Phytochemical evaluations
- Trial batches for Transdermal patches (extract : polymer in different ratio)
- Evaluations

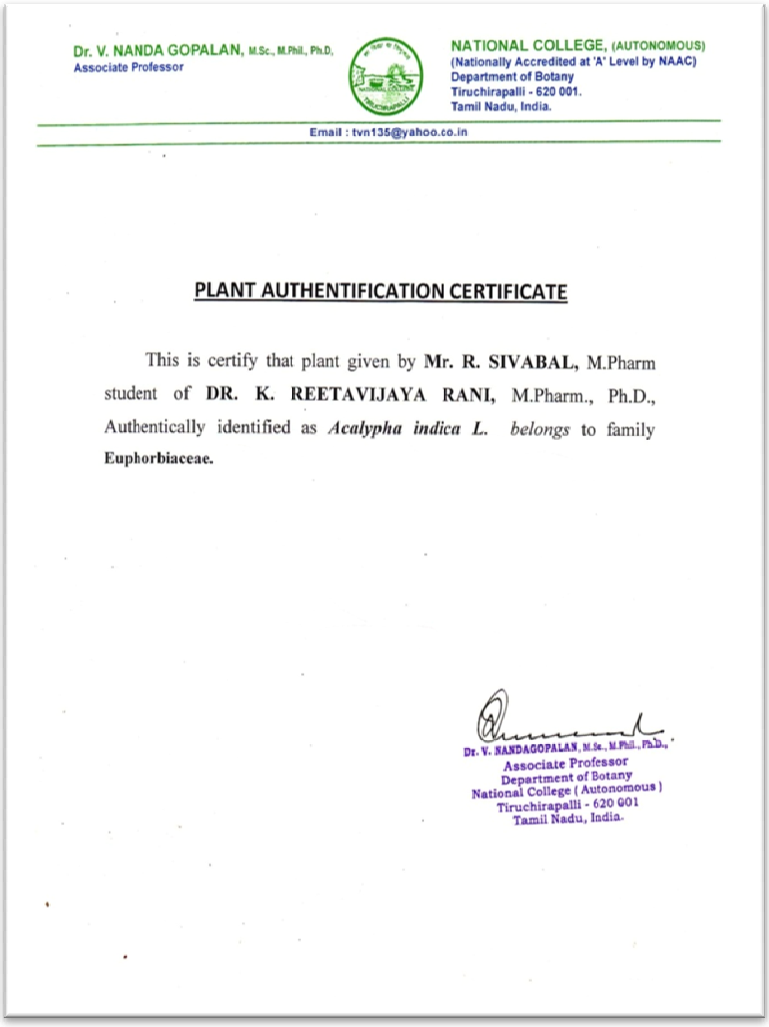
1) Physico chemical evaluation

- ✓ Percent moisture absorption
- ✓ Percent moisture loss
- ✓ Thickness
- ✓ Weight variation
- ✓ Folding endurance
- ✓ Surface pH
- ✓ Drug content

2) *In- vitro* Evaluation

- Stability studies
- Anti-Microbial studies
- *Ex-vivo* studies
- Release kinetics
- Results and discussions
- Summary and Conclusion

PLANT AUTHENTICATION CERTIFICATE



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Department of Botany
Tiruchirapalli - 620 001.
Tamil Nadu, India.

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PLANT AUTHENTIFICATION CERTIFICATE

This is certify that plant given by **Mr. R. SIVABAL**, M.Pharm student of **DR. K. REETAVIJAYA RANI,** M.Pharm., Ph.D., Authentically identified as ***Acalypha indica L.*** *belongs* to family **Euphorbiaceae.**

Dr. V. NANDAGOPALAN, M.Sc., M.Phil., Ph.D.,
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5. PLANT DESCRIPTION

Fig No.12: Leaves of *Acalypha indica* Linn.

5.1.1 *Acalypha indica* Linn.[37]

Kingdom	**:**	Plantae
Order	**:**	Malpighiales
Family	**:**	Euphorbiaceae
Genus	**:**	*Acalypha*
Species	:	*indica*
Common Nam	**:**	*Acalypha indica* Linn.
English	**:**	Indian acalypha, Indian nettle, three-seeded mercury
Tamil	**:**	Poonamayakki, Kuppaimeni
Sanskrit	**:**	Harita-manjari
Parts Used	**:**	Leaf

5.1.2.Description

Colour **:** Green

Taste **:** Bitter

Odour **:** Pungent

Solubility **:** Methanol

5.1.3.Distribution

India, Africa, Oceania, and South East Asia

5.1.4.Habitat

Common annual shrub in Indian gardens, backyards of houses and waste place throughout the plains of India

5.1.5.Vernacular names of *Acalypha indica* Linn.

English : Indian Acalypha

Kannada **:** Chalmari

Malayalam **:** Kupameni

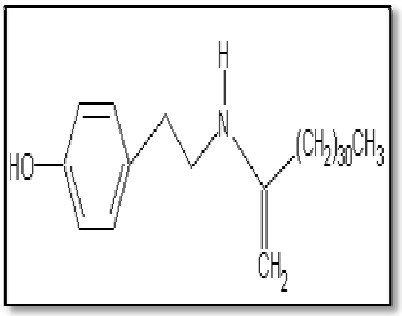
Others **:** Rudra, kuppu, khokali

Tamil **:** Kuppaimeni**,** naaikurungu, poonamayakki

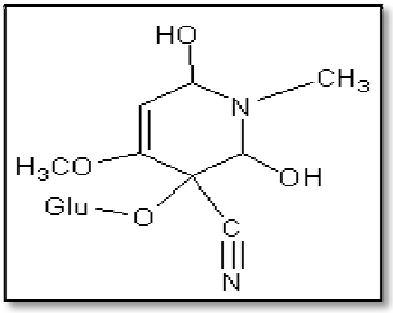
5.1.6. Chemical constituents

- ✓ Acalyphamide
- ✓ Acalyphin
- ✓ Acalyphamide acetate
- ✓ Alkaloid
- ✓ Catachols
- ✓ Flavonoids

- ✓ Phenolic compounds
- ✓ Saponins and steroids
- ✓ Volatile oil and fatty acids
- ✓ Kaempferol glycosides
- ✓ Mauritianin
- ✓ Clitorin
- ✓ Quercitrin
- ✓ Hesperitin



ACALYPHAMIDE



ACALYPHIN

5.1.7.Pharmacological activity[38]

- ✓ Antiulcer activity
- ✓ Anti malarial activity
- ✓ Anti-tuberculosis activity
- ✓ Analgesic and anti inflammatory activity
- ✓ Anti diabetic activity
- ✓ Diuretic activity
- ✓ Antimicrobial activity
- ✓ Antibacterial activity
- ✓ Alpha amylase inhibitory activity

5.1.8.Traditional uses

- ✓ Anti-inflammatory, analgesic, irritations, stabbing pain, wheezing
- ✓ Anti-tuberculosis, burns
- ✓ For removing the toxin arising out of Rat bite
- ✓ Mixed with garlic they are used as Anthelmintic in worms
- ✓ Mixed with garlic they are applied to scabies (MateriaMedica (Vegetable section), Volume I, by Dr. MurugesaMuthaliar, pages: 359, publisher, Tamilnadu Siddha Medical Council, Chennai. Fourth edition 1988.)

5.2. EXCIPIENT PROFILE:

5.2.1.Pectin:[39]

Pectin is structural hetero-polysaccharides contained in the primary cell walls of terrestrial plants. Pectin is a naturally occurring biopolymer that is finding increasing applications in the pharmaceutical and biotechnology industry. It has been used successfully for many years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabilizer. Pectin also has several unique properties that have enabled it be used as a matrix for the entrapment and/or delivery of a variety of drugs, proteins and cells. This review will first describe the source and production, chemical structure and general properties of pectin. The methods of gel formation and properties of the gels will then be discussed.

Physical properties : White to light brown powder

Chemical Structure :

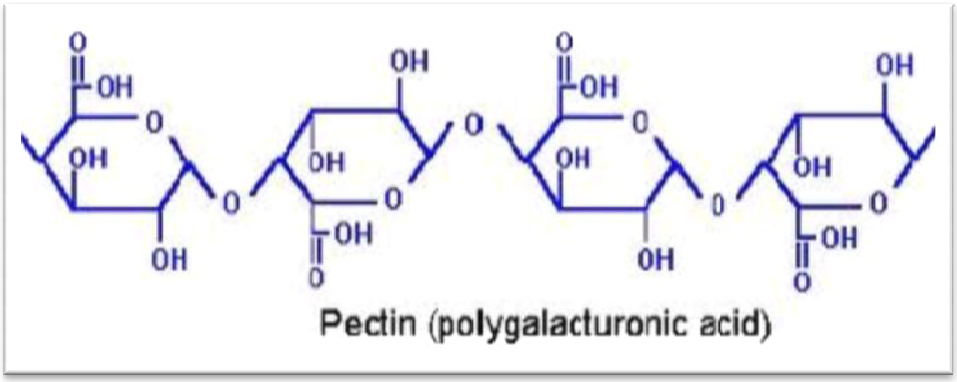


Fig No. 13: Pectin

Molecular weight : 60-130,000g/ml

Uses : Gelling agent, thickening agent, stabilizer in food

5.2.2. Sources of Pectin

- Apples – 1 to 1.5%
- Apricot – 1%
- Cherries – 0.4%
- Oranges – 0.5 to 3.5%
- Carrots approx – 1.4%
- Citrus peels – 53%

5.2.3. General properties of Pectin

Pectin is a natural polymer. Pectin is soluble in pure water. Monovalent cation (alkali metal) salts of pectinic and pectic acids are usually soluble in water, di- and trivalent cations salts are weakly soluble or insoluble. Dry powdered pectin, when added to water, has tendency to hydrate very rapidly, forming clumps. These crumps consist of semi dry packets of pectin contained in an envelope of highly hydrated outer coating. Further solubilisation of such cramps is very slow. Dilute pectin solutions are Newtonian but a moderate concentration, they exhibit the non-Newtonian, pseudoplasticbehaviourcharacterstics. As with solubility, the viscosity of a pectin solution is related to the molecular weight, concentration of the preparation, and the pH and presence of counter ions in the solution. Viscosity, solubility and gelation are generally related. For example, factors that increase gel strength will increase the tendency to gel, decrease solubility, and increase viscosity, and vice versa.

5.2.4. Pharmaceutical uses of pectin

- ✓ Pectin has applications in the pharmaceutical industry. Pectin favorably influences cholesterol levels in blood. Consumption of at least 6g/day of pectin is necessary to have a significant effect in cholesterol reduction.
- ✓ Pectin acts as a natural prophylactic substance against poisoning with toxications. It has been shown to effective in removing lead and mercury from the gastrointestinal tract and respiratory organs.
- ✓ Pectin injected intravenously, shortens the coagulation time of drawn blood, thus being useful in controlling hemorrhage or local bleeding.

- ✓ Pectin and combinations of pectin with other colloids have been used extensively to treat diarrheal diseases, especially in infants and children.
- ✓ Pectin reduces rate of digestion by immobilizing food components in the intestine. This results in less absorption of food.
- ✓ Pectin hydrogels have been used in tablet formulations as a binding agent matrix tablet formulations. HM-pectin's for their potential value in controlled release matrix formulations. The application of a binary polymer system, (i.e) HM-pectin and hydroxypropyl methylcellulose.
- ✓ Pectin beads prepared by the ionotropic gelation method were used as a sustained release drug delivery system.
- ✓ Pectin has a promising pharmaceutical uses and is presently considered as a carrier material in colon-specific drug delivery systems (for systemic action or a topical treatment of diseases such as ulcerative colitis, crohn's disease, colon carcinomas)
- ✓ Pectin is an interesting candidate for pharmaceutical use, e.g. as a carrier of variety of drugs for controlled release applications. Many techniques have been used to manufacture the pectin-based delivery systems, especially ionotropic gelation and gel coating.

5.3. Sodium alginate:[40]

Nonproprietary Names:

BP **:** Sodium alginate

PhEur **:** Natriialginas

USPNF **:** Sodium alginate

Synonyms

Algin; alginic acid, sodium salt; E401; *Kelcosol; Keltone; Protanal;* sodium polymannuronate

5.3.1. Structure of Sodium alginate

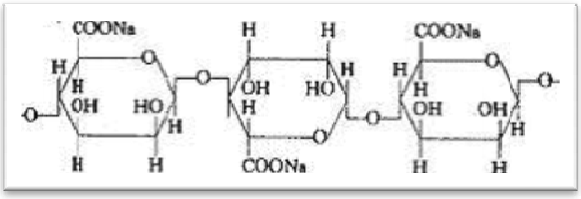


Fig No.14: Sodium alginate

Chemical Name and CAS Registry Number

Sodium alginate [9005-38-3]. Sodium salt of alginic acid, which is a mixture of polyuronic acids composed of residues of D-mannuronic acid and L-guluronic acid. The block structure and molecular weight of sodium alginate samples have been investigated

5.3.2. Functional Category

Stabilizing agent; suspending agent; tablet and capsule disintegrant; tablet binder; viscosity increasing agent.

5.3.4. Applications in Pharmaceutical Formulation or Technology

Sodium alginate is used in a variety of oral and topical pharmaceutical formulations. In tablet formulations, sodium alginate may be used as both a binder and disintegrant; it has been used as a diluent in capsule formulations. Sodium alginate has also been used in the preparation of sustained-release oral formulations since it can delay the dissolution of a drug from tablets, capsules, and aqueous suspensions.

In topical formulations, sodium alginate is widely used as a thickening and suspending agent in a variety of pastes, creams and gels and as a stabilizing agent for oil-in-water emulsions. Recently, sodium alginate has been used for the aqueous microencapsulation of drugs, in contrast with the more conventional microencapsulation techniques which use organic solvent systems. It has also been used in the formation of nanoparticles.

The adhesiveness of hydrogels prepared from sodium alginate has been investigated and drug releases from oral mucosal adhesive tablets, and buccal gels, based on sodium alginate have been reported. Other novel delivery systems containing sodium alginate include ophthalmic solutions that form a gel *in-situ* when administered to the eye; and *in- situ* forming gel containing paracetamol fororal administration; and a freeze-dried device intended for the delivery of bone-growth factors Hydrogel systems containing alginates have also been investigated for delivery of proteins and peptides.

Therapeutically, sodium alginate has been used in combination with an H2-receptor antagonist in the management of gastro esophageal reflux, and as a haemostatic agent in surgical dressings. Alginate dressings, used to treat exuding wounds, often contain significant amounts of sodium alginate as this improves the gelling properties. Sponges composed of sodium alginate and chitosan produce a sustained drug release and may be useful as wound dressings or as tissue engineering matrices. Sodium alginate is also used in cosmetics and food products.

5.3.5. Description

Sodium alginate occurs as an odorless and tasteless, white to pale yellowish-brown coloured powder.

5.3.6. Typical Properties

Acidity/alkalinity: pH 7.2 for a 1% w/v aqueous solution.

Solubility

Practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than 30%. Also, practically insoluble in other organic solvents and aqueous acidic solutions in which the pH is less than 7. Slowly soluble in water, forming a viscous colloidal solution.

Viscosity (dynamic)

Various grades of sodium alginate are commercially available that yield aqueous solutions of varying viscosity. Typically, a 1% w/v aqueous solution, at

20^0C, will have a viscosity of 20-400 m Pas (20-400cP). Viscosity may vary depending upon concentration, pH, temperature, or the presence of metal ions. Above pH 10, viscosity decreases.

5.3.7. Stability and Storage Conditions

Sodium alginate is a hygroscopic material, although it is stable if stored at low relative humidities and a cool temperature.

Aqueous solutions of sodium alginate are most stable at pH 4-10. Below pH 3, alginic acid is precipitated. A 1% w/v aqueous solution of sodium alginate exposed to differing temperatures had a viscosity 60-80% of its original value after storage for 2 years. Solutions should not be stored in metal containers.

Sodium alginate solutions are susceptible on storage to microbial spoilage, which may affect solution viscosity. Solutions are ideally sterilized using ethylene oxide, although filtration using a 0.45µm filter also has only a slight adverse effect on solution viscosity. Heating sodium alginate solutions to temperatures above 70^0C causes depolymerization with a subsequent loss of viscosity. Autoclaving of solutions can cause a decrease in viscosity, which may vary depending upon the nature of any other substances present. Gamma irradiation should not be used to sterilize sodium alginate solutions since this process severely reduces solution viscosity.

Preparations for external use may be preserved by the addition of 0.1% chlorcresol, 0.1% chloroxylenol or parabens. If the medium is acidic, benzoic acid may also be used. The bulk material should be stored in an airtight container in a cool, dry place.

Incompatibilities

Sodium alginate is incompatible with acridine derivatives, crystal violet, phenyl mercuric acetate and nitrate, calcium salts, heavy metals, and ethanol in concentrations greater than 5%. Low concentrations of electrolytes cause an increase in viscosity but high electrolyte concentrations cause salting-out of sodium alginate;

Safety

Sodium alginate is widely used in cosmetics, food products, and pharmaceutical formulations, such as tablets and topical products, including wound dressings. It is generally regarded as a nontoxic and non irritant material, although excessive oral consumption may be harmful. A study in five healthy male volunteers fed a daily intake of 175 mg/kg body-weight of sodium alginate for 7 days, followed by a daily intake of 200 mg/kg body-weight of sodium alginate for a further 16 days, showed no significant adverse effects.

The WHO has not specified an acceptable daily intake for alginic acid and alginate salts as the levels used in food do not represent a hazard to health.

Inhalation of alginate dust may be irritant and has been associated with industrial-related asthma in workers involved in alginate production. However, it appears that the cases of asthma were linked to exposure to seaweed dust rather than pure alginate dust.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Sodium alginate may be irritant to the eyes or respiratory system if inhaled as dust. So Eye protection, gloves and a dust respirator are recommended. Sodium alginate should be inhaled in a well-ventilated environment.

5.4. Hydroxy propyl methyl cellulose(HPMC) :[41]

5.4.1. Chemical structure

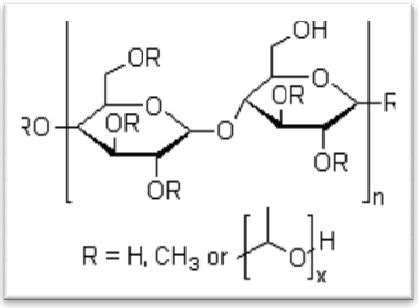


Fig No.15: HPMC

Emperical formulae	**:**	$C_{12}H_{20}O_{10}$
Synonyms	**:**	Hypromellose, Methocel 856N, Metolose, Accel R 100, Methyl hydroxypropyl cellulose, 2-hydroxypropyl cellulose methyl ether
Molecular weight	**:**	324.2848
Non-Proprietary Names	**:**	
USP, BPC	**:**	Hydroxy Propyl Methyl Cellulose-2208,2906,2910; Hypromellose
5.4.2. Molecular weight	**:**	Approx.86,000
5.4.3. Functional category	**:**	Suspending and/or viscosity-increasing agent; Tablet binder, coating agent, adhesive anhydrous ointment, ingredient
5.4.4. Description	**:**	White to off-white powder, odourless,
5.4.5. Apparent density	**:**	0.250-0.70g/cm^3

5.4.6. Solubility	**:**	HPMC can be soluble in water and some organic solvents, as the right proportion of ethanol/water, propy alcohol/water, ethylene dichloride, etc.

5.4.7. Pharmacopoeial Specifications

Specific gravity	**:**	1.26-1.31
pH	**:**	6.0-8.0

5.4.8. Pharmaceutical Application

- Film former in tablet film coating.
- Lower viscosity grades are used in aqueous film coating and higher viscosity grades are used in solvent film coating.
- High viscosity grades are used to retard the release of water soluble drugs.
- Thickening agent added to vehicles for eye drops and artificial tear solutions at 0.45-1.0% concentrations.
- Protective colloid which prevents droplets and particles from coalescing or agglomerating, thus inhibiting the formation of sediments, emulsifier, suspending agent and stabilizer in gels and ointments.
- Adhesive in plastic bandages.

5.4.9. Safety

Human and animal feeding studies have shown hydroxyl propyl methyl cellulose to be safe.

5.4.10. Stability and storage

Very stable in dry conditions. Solutions are stable at pH 3 - 11. Store in a tight container, in a cool place.

5.5.Glycerin

5.5.1.Nonproprietary Names

BP : Glycerol.

PhEur : Glycerolum.

5.5.2. Synonyms

Croderol; E422; glycerine; Glycon G-100; Kemstrene; Optim; Pricerine; 1,2,3- propanetriol; trihydroxypropane glycerol.

5.5.3. Chemical name

Propane 1, 2, 3-triol.

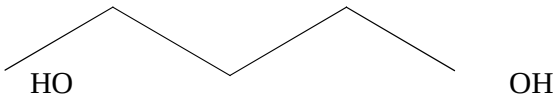
5.5.4. Empirical Formula

$C_3H_8O_3$

5.5.5.Molecular Weight

92.09

5.5.6. Structural Formuula



OH

GLYCEROL

5.5.7. Description

Itis a clear, colorless, odourless, viscous, hygroscopic liquid, it has a sweet taste, approximately 0.6times as sweet as sucrose.

5.5.8. Functional category

Antimicrobialpreservative,emollient,humectant,plasticizer,solvent.

5.5.9.Typical properties:

- **Melting point** : 17.8^0c
- **Solubility** **:** soluble in water,methanol
-

5.5.10.Applications in Pharmaceutical Technology:

It is used in a wide variety of pharmaceutical formulations including oral, otic, ophthalmic, topical & parenteral preparations. In topical pharmaceutical formulations and cosmetics, it is used primarily for its humectant & emollient properties. Inparenteral formulations it is used mainly as a solvent. In oral solutions, glycer in is used a solvent, sweetening agent, antimicrobial preservative and viscosity increasing agent. It is also used as a plasticizer and in film coatings. It is additionally used in topical formulation such as creams and emulsions. It is used as a plasticizer of gelatin in the production of soft-gelatin capsules and gelatin suppositories. It is employed as a therapeutic agent in a variety of clinical applications, and is also used as a food additive.

5.5.11.Stability and Storage Conditions:

It is hygroscopic. Pure glycerinis not proneto oxidation bythe atmosphere under ordinary storage conditions but it decomposes on heating, with the evalution of toxic acrolein. Mixture of glycerin with water, ethanol & propyleneglycol are chemically stable.It may crystallize if stored at low temperatures; the crystals do not melt until warmed to 20^{0C}. It should be stored in an airtight container, in a cool, dry place.

5.6. Dimethyl Sulfoxide

5.6.1. Non-proprietary Names

BP : Dimethyl sulfoxide

PhEur : Dimethylissulfoxidum

USP : Dimethyl sulfoxide

5.6.2. Synonyms

Deltan; dimexide; dimethyl sulphoxide; DMSO; Kemsol; methylsulfoxide; Rimso-50; sulphinyl bismethane

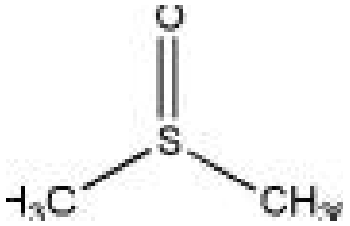
5.6.3. Chemical Name and CAS Registry Number

Sulfinyl bismethane [67-68-5]

5.6.4. Empirical Formula and Molecular Weight

C_2H_6OS M.W: 78.13

5.6.5. Structural Formula



5.6.6. Functional Category

Penetration enhancer; solvent.

5.6.7. Applications in Pharmaceutical Formulation or Technology

Dimethyl sulfoxide is a highly polar substance that is aprotic, therefore lacking acidic and basic properties. It has exceptional solvent properties for both organic and inorganic components, which are derived from its capacity to associate with both ionic species and neutral molecules that are either polar orpolarizable. Dimethyl sulfoxide enhances the topical penetration of drugs owing to its ability to displace bound water from the stratum corneum; this is accompanied by the extraction of lipids and configurational changes of proteins.The molecular interactions between dimethyl sulfoxide and the stratum corneum, as a function of depth and time, have been described. Much of the enhancement capacity is lost if the solvent is diluted. Increases in drug penetration have been reported with dimethyl sulfoxide concentrations as low as 15%, but significant increases in permeability generally require concentrations higher than 60–80%. Furthermore, while low molecular weight substances can penetrate quickly in to the deep layers

of the skin, the appreciable transport of molecules with amolecular weight of more than 3000 is difficult. The use of dimethyl sulfoxide to improve transdermal delivery has been reported forcyclosporin,timolol, and a wide range of other drugs. Dimethyl sulfoxide has also been used in theformulation of an injection containing allopurinol. It has also been investigated for use in an experimental parenteral preparation for the treatment of liver tumors.In paint formulations of idoxuridine, dimethyl sulfoxide acts both as a solvent to increase drug solubility and a means of enabling penetration of the antiviral agent to the deeper levels of the epidermis.

5.6.8. Typical Properties

Boiling point:

189°C

Dielectric constant:

48.9 at 20°C

Solubility:

Miscible with water with evolution of heat; also miscible with ethanol(95%), ether and most organic solvents; immiscible with paraffins, hydrocarbons. Practically insoluble in acetone, chloroform, ethanol (95%), and ether.

Vapor pressure:

0.37 mm at 20°C

Viscosity (dynamic):

1.1 mPa s (1.1 cP) at 27°C

5.6.9. Stability and Storage Conditions

Dimethyl sulfoxide is reasonably stable to heat but upon prolonged reflux it decomposes slightly to methyl mercaptan and bismethylthiomethane. This decomposition is aided by acids, and is retarded by many bases. When heated to decomposition, toxic fumes are emitted.

At temperatures between 40–60°C, it has been reported that dimethyl sulfoxide suffers a partial breakdown, which is indicated by changes in physical properties such as refractive index, density, and viscosity. Dimethyl sulfoxide should be stored in airtight, light-resistant containers.The PhEur 2005 states that glass containers should be used. Contact with plastics should be avoided.

6. MATERIALS AND METHODS

6.1. List of Chemicals

Table No: 2

S.NO	Chemical Name	Company Name
1	SLS	Merck Limited, Mumbai
2	Pectin	Loba, Mumbai
3	Sodium alginate	Kemphasol, Bombay
4	HPMC	HiMedia Laboratories Limited, Mumbai
5	DMSO	Merck Limited, Mumbai
6	Glycerin	Merk Limited, Mumbai
7	Potassium dihydrogenortho phosphate	Microfine Chemicals, New Delhi
8	Sodium hydroxide	Microfine Chemicals, New Delhi

6.2. List of Equipments

Table No. 3:

S.NO	Name of Equipment	Name of Manufacturer	Purpose
1	Dessicator	-	Moisture content studies
2	Digital Verniercaliper	-	Patch thickness Studies
3	Electronic balance	Sortorius, Germany	Weighing purpose
4	Digital pH meter	Elico Ltd, AP, India	Surface pH study
5	Fourier transform Infrared Spectrometer	Perkin-Elmer, USA	Compatibility studies
6	Magnetic Stirrer	ROTEK, W.Vengola, Kerala, India	Diffusion Studies
7	UV Spectrophotometer	Shimadzu 1700, Japan	Finding Absorption maxima
8	Environment test Chamber	Heco, Germany	For stability studies

6.3. Preformulation Studies

Preformulation testing is the first step in the rational development of dosage forms of drugs substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be man produced.

The following Preformulation studies are carried out

- Finding the absorption maxima
- Physical appearance
- Solubility
- Melting point
- Standard curve
- Infrared spectroscopy studies (compatibility studies)

6.4. Finding the absorption maxima (λ max):

The absorption maxima were found for identification of drug.

Ultraviolet Visible spectrophotometry has been used to obtain specific information on the chromophoric part of the molecules. Organic molecules in solutions when exposed to light in the Visible/Ultraviolet region of the spectrum absorb light of particular wavelength depending on the type of electronic transition associated with the absorption.

The drug solution (5, 10, 15, 20, 25 µg/ml) in distilled water was taken in a standard cuvette and scanned in the range of 200-400 nm in UV spectrophotometer. It exhibits maxima at 260nm. Therefore, further all measurements were taken at 260nm.

6.5.Collection of *Acalypha indica* Linn. leaves

Acalypha indica Linn. leaves were collected from in and around Trichy district, Tamilnadu. Collected leaves were authenticated by Botanist, Dept. of Botany, National College, Trichy. Leaves were cleaned and shade driedat room temperature.

6.6. Extraction of leaves of *Acalypha indica Linn.*[42]

The shade dried leaves were subjected to size reduction and passed in to sieve no 20 and then 40.About 500g of the dried powder was extracted continuously in Soxhlet apparatus with petroleum ether for 24 hrs to remove the waxy materials. Then it was extracted with Methanol for 72hrs.After 72hrs, the solvent was evaporated to obtain the crude extract (6.5%w/v). The extract was dried under vacuum oven.

Fig No.16: Soxhylation

6.7. Phytochemical Studies[43]

The ME (Methanolic Extract) was subjected to phytochemical studies to find out the presence and absence of constituents.

Table No.4: Phytochemical Tests

Test for Alkaloids

EXPERIMENT	OBSERVATION	INFERENCE
1.Dragendroff's test: The extract was treated withDragendroff's reagent (potassium bismuth iodide solution)	Orange brown Precipitate was formed	Presence of alkaloids
2.Mayers' reagent's: The extract was treated with Mayer's (potassium mercuric iodide solution) reagent	Precipitate formed	Presence of alkaloids
3.Wagner's reagent: The extract was treated with wagner's reagent (iodide and potassium triiodide solution)	Reddish brown Precipitate was formed	Presence of alkaloids

Test for Glycosides

1.Brontragers test: To the extract add dilute H_2SO_4 and filtered. Filtrate was extract with little chloroform layer was separated out and add equal volume of dilute NH_3.	Red colourobserved in ammonical layer	Presence of glycosides

Test for Saponin glycosides

1.Foam test: Shake the extract with water	Foam was produced/formed	Presence of saponin Glycosides

Test for Tannins and Phenolic compounds

1.Ferric chloride test: To the aqueous extract few drops of ferric chloride solution were added	Dark black colour formed	Presence of tannins and phenolic compounds
2.Bromine water test: To the aqueous extract is treated with bromine water	Discoloration of bromine Water	Presence of tannins and phenolic compounds
3.$KMnO_4$test: To the aqueous extract is treated with dilute $KMnO_4$	Discoloration of solution	Presence of tannins and phenolic compounds

Test for Reducing sugar

1.Benedict's test: 0.5ml of extract solution 1ml of water 5 to 8 drops of fehlings solution was added	No brick red precipitate	Absence of reducing sugar

Test for Amino acids

1.Ninhydrin test: The aqueous extract is heated with 5%ninhydrin solution on boiling water bath for 10 min.	No purple coloured	Presence of amino acids
2.The aqueous extract is treated with solution sodium hydroxide and lead acetate solution and boiled	No black precipitate is formed	Presence of amino acids

Test for Flavonoids

1. **Shinoda test:** To the methanol extract add potassium hydroxide solution and then 10% ammonia.	Yellow colour Precipitate formed.	Presence of flavonoids
2.To the ethanol extract, add few drops of Lead acetate solution.	Yellow colour Precipitate formed.	Presence of flavonoids.

Test for Terpenoids

1.4gm of extract was treated with 0.5ml of acetic anyhydride and 0.5ml of chloroform and added concentrated solution of sulphuric acid	No Red violet colour was obtained	Absence of terpenoids

Test for Steroids

1.Libermann- Buchard Test: To extract add chloroform solution a few drops of acetic anhydride and 1ml of con. H_2SO_4 were added through the side of the test tube and set aside for a while.	Brown ring was formed at the junction.	Presence of steroids
2. Salkowski Test: To the extract add chloroform solution few drops of con. H_2SO_4 was added shaken and allowed to stand.	Greenish fluorescence was formed.	Presence of steroids
3. Libermann's Reaction: Mix 3ml of extract with 3ml of acetic anhydride, heat and cool. Add few drops of Con. H_2SO_4.	Blue colour was formed	Presence of steroids

6. 8.Preparation of Transdermal Patch[44]

Three batches of drug loaded (ME of *Acalypha indica* Linn.) transdermal patches were prepared using drug with different polymer ratio (1:1, 1:2, and 1:4). Weighed quantity of polymer was dissolved in calculated quantity of water and heated on a water bath. Calculated amount of extract was added to the above mixture and stirred well until a homogenous mixture was formed. Then calculated amount of permeation enhancer and Glycerin were added.

The resultant mixture was poured into a petridish and air dried at room temperature for 24hrs.The patches are then peeled off from the petridish with the help of a knife and kept in desiccator.

Calculation

Diameter of glass plate = X cm

Radius of glass plate = Y cm

Area of glass plate = πr^2 (π=3.14)

= 3.14 × (y)

= Z cm^2

Z/X= Area/diameter = capacity (ml)

6.9.Calibration Curve of Methanolic Extract of *Acalypha indica* Linn.

6.10. Preparation of phosphate buffer pH 7.4

Phosphate buffer pH 7.4 was prepared as per the method described in I.P 1996 using disodium hydrogen phosphate and sodium hydroxide. The pH was adjusted to 7.4 prior to quantitative estimation.

6.11. Preparation of calibration curve of *Acalypha indica* Linn. extract in phosphate buffer pH 7.4

Accurately weighed quantity (100mg) of MEA was transferred into a 100ml volumetric flask and dissolved in small amount of phosphate buffer (pH 7.4) and made upto the volume with same buffer to make the standard stock solution of 1 mg/ml.

From the stock, 1ml was taken in 10ml volumetric flask and made up the volume with the buffer; from this solution 0.5ml to 3ml solution was transferred to 10ml volumetric flask and made upto required volume with more buffer and the resulting concentration ranges from 5 to 50 µg/ml. The absorbance of these solutions was determined at 260nm using UV spectrophotometer.The calibration curve was constructed between the absorbance and concentration.

6.12. Physico chemical evaluation of *Acalypha indica* Linn. Transdermal patch:[45,46,47,66,67]

Formulated patches were subjected to the preliminary evaluation tests. Patches with any imperfections, entrapped air, or differing in thickness, weight (or) content uniformity were excluded from further studies.

1. Uniformity of weight

This was done by weighing five different patches of individual batch taking the uniform size at random and calculating the average weight of three. The tests were performed on patch which was dried at 60˚C for 4 hrs prior to testing. The result is shown in Table No:18

2. Thickness of the Patch

The thickness of the patch was assessed by using digital vernier caliper at different points of the patch. From each formulation three randomly selected patches were used. The average value for thickness of a single patch was determined.[61] The results are shown in TableNo: 19

3.Drug content determination

The patch were taken and added to a beaker containing 100 ml of Phosphate buffer saline pH 7.4. The medium was stirred magnetic bead for 5 hrs.The solution was later filtered and analyzed for drug content with proper dilution at 260 nm spectrophotometrically. The results are shown in Table No: 20

4. Folding Endurance

This was determined by repeatedly folding one patch at the same place till it broke. The number of times the patch could be folded at the same place without breaking gave the value of folding endurance. The result shown in Table No :21

5. Percentage Moisture uptake:

The patch were weighed accurately and placed in desiccators containing aluminiumchloride. After 24 hrs, the film were taken out and weighed. The percentage moisture uptake was calculated as the difference between final and initial weight. With respect to initial weight. It is calculated by using following formula.[54]

$$\text{Percentage moisture content} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

The results are shown in Table No: 22

6. Percentage Moisture content [68]

The patch were weighed and kept in desiccators containing calcium chloride. After 24hrs the patch were taken out and weighed. The percentage moisture content was calculated using the following formula.

$$\text{Percentage moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

The result shown in Table No: 23

7.Determination of surface pH [69]

The patches were allowed to swell by keeping them in contact with 1 ml of distilled water for 2 h at room temperature and pH was noted down by bringing the electrode in contact with the surface of the patch, allowing it to equilibrate for 1 min. The result are shown in Table No:24

8. Percent Elongation [70]

When stress is applied, a patch sample stretches and this is referred to as strain. Strain is basically the deformation of patch divided by original dimension of the sample. Generally elongation of patch increases as the plasticizer content increases. It is calculated by using following formula.

$$\text{Percentage elongation} = \frac{\text{Increase in length of patch}}{\text{Initial length of patch}} \times 100$$

The result shown in Table No: 25

9. Tensile strength

Tensile strength is the maximum stress applied to a point at which the patch specimen breaks . It is calculated by the applied load at rupture divided by the cross-sectional area of the strip as given in the equation below:

$$\text{Percentage elongation} = \frac{\text{Load at failure}}{\text{Patch thickness x Patch width}} \times 100$$

The result shown in Table No: 26

Cellophane Membrane Treatment:[48]

Cellophane membrane was boiled in the distilled water for 1 hr and washed with fresh distilled water for three times and kept in ethanol for 24 hrs. It was washed with distilled water and treated with 0.3% sodium sulphite and soaked in distilled water for 2 min at 60°C followed by acidified with 0.2% sulphuric acid. Finally the membrane was dipped in boric buffer (pH 9) till it is used for permeation study.

Drug Permeation Studies:[49]

The *in-vitro* release rate of *Acalypha indica* Linn. transdermal patch were evaluated by open ended tube through using PB pH 7.4 as diffusion medium up to 12 hours studies. The cellophane membrane is tided in one end of the tube and then immersed in the receptor compartment containing 400ml of PB pH7.4.Which was stirred at medium speed and maintained at 37°C±2°C. Samples were withdrawn at regular time intervals and the same volume was replaced by fresh diffusion medium. (79) The samples were analyzed using UV – visible spectrophotometer (Shimadzu UV1700) set at 260 nm. The result is shown in Table No:30 and Fig.No:29

6.13.Experimental conditions of *ex- vivo* transdermal permeation study of H4 formulation [54,55,56,57,58,59,60]

Ex-vivo Transdermal permeation studies carried out using Goat abdomen skin

- The receptor compartment consisted of 400ml of Phosphate buffer (pH 7.4) in 500 ml beaker
- Temperature was maintained at 37 ± 0.5°C and stirred at 900 rpm
- The *Acalypha indica* Linn.Transdermal patch was placed in Goat abdomen skin and tied to the one end of open-ended glass cylinder that was then dipped into freshly prepared phosphate buffer on magnetic stirrer
- Samples were taken from receptor medium at 0, 5, 10, 15, 30, 45, 60, 120, 180, 240 and 300, 360, 420,480 , 540, 600, 660, 720 min
- Periodically 5ml of sample was withdrawn and same volume of medium was replaced with fresh buffer
- All the Samples were assayed spectrophotometrically at 260 nm using PB 7.4 pH as blank

The result shown in Table No: 31 and Fig No:33

6.14. Release Kinetics [60,61,62,63,64,65]

Data obtained from *in-vitro* release studies were fitted to various kinetic equations. The kinetic models used are zero order equations (Q=k0t), First order equation {ln (100 – Q) =ln Q – klt}, Higuchi equation (Q=kt1/2), Hixson and crowell model Qt1/3 Vs t and Qt2/3 Vs – Modified root cube equation. Further, to find out the mechanism of drug release, first 60% drug release was fitted in Korsmeyer and Peppas equation (Q=kptn). Where, Q is the percent of the drug release at time t and k0 and kt are the coefficients of the equations and ‘n’ are the release exponent. The ‘n’ value is used to characterize different release mechanism.

The order of drug release can be assessed by graphical treatment of drug release data.

A plot of cumulative % drug release versus time would be linear if the drug release follows zero order (i.e. Concentration independent release).

A plot of log of % remaining drug versus time would be linear, if the drug release follows first order (i.e. Concentration dependent release)

The linear equation for zero order drug release plot is:

$$Ct = C0 - Kt$$

Where,

Ct = concentration remaining at time t,

Co = original concentration,

t = time,

K = release rate

The linear equation for first order release plot is

$$\text{Log } C = \frac{\log C0 - Kt}{2.303}$$

A matrix device as the name implies, consists of drug dispersed homogeneously throughout a polymer matrix

In this model, drug in the outside layer exposed to the bathing solution is dissolved first and then diffuses out of the matrix. This process continues with the interface between the bathing solution and the solid drug moving towards the interior. Obviously, for this system to be diffusion controlled, the rate of dissolution of drug particles within the matrix must be much faster than the diffusion rate of dissolved drug leaving the matrix.

Hydrophilic matrix tablets contain a water swellable polymer. On

$$[1 - Mt / M] 1/3 = 1\text{-}kt$$

Where,

Mt = mass of drug release at time t,

M = mass release at the infinite time,

K = rate of erosion,

t = time

Thus a plot of [1 – Mt / M] 1/3 versus the time will be linear. If the release of drug from the matrix is erosion controlled.

In order to ascertain whether the drug release occurs by diffusion or erosion, the drug release data was subjected to following modes of data treatments.

1) Amount of drug release versus square root of time (Higuchi Plot).

2) [1 – Mt / M] 1/3 versus time.

6.15. Stability studies:

a) Stability:[50,51,71]

Stability is official defined as the time lapse during which the drug product retains the same property and characteristics that it possessed at the time of manufacture. This process being early development phases.

Instability in modern formulation is often undetectable only after considerable storage period under normal conditions. To assess the stability of a formulated product it is usual to expose it to high stress conditions to enhance deterioration and therefore the time required for testing is reduced. Common high stresses are temperature and humidity. This will eliminate unsatisfactory formulation.

b) Strategy of stability testing:

- The study of drug decomposition kinetics
- The development of stable dosage form
- Establishment of expiry date for commercially available drug product is some of the needs of stability testing
- Data from which study should be provided on at least 3 primary batches of the drug product
- The batches should be manufactured to a minimum of pilot scale
- Important point of view of the safety of the patient, patient relieves a uniform dose of drug throughout the shelf life of the product

Table No.5: Stability condition chart

Intended Storage Condition	StabilityTest Method	ICH Test Temperature and Humidity (period in months)	WHO Test Temperature and Humidity(period in Months)
Room Temperature	Long term	25±2^{0}C/60±5%RH	25±2^{0}C/60±5%RH or 30±2^{0}C/65±5%RH 30±2^{0}C/75±5%RH
	Intermediate	30±2^{0}C/65±5%RH	30±2^{0}C/65±5%RH
	Accelerated	40±2^{0}C/75±5%RH	40±2^{0}C/75±5%RH
Refrigerated	Long term Accelerated	5^{0}C/ambient 25±2^{0}C/60±5%RH	5±3^{0}C 25±2^{0}C/60±5%RH or 30±2^{0}C/65±5%RH
Freezer	Long term	-20^{0}C/ambient	-20^{0}C±5^{0}C

6.16. Screening of Antimicrobial activity of *Acalypha indica* Linn.

6.16.1. Anti-Bacterial Activity[52,53]

Principle

Discs impregnated with known concentration of antibiotics discs are placed on agar plate that has been inoculated (or) seeded uniformly over the entire plate with a culture of the bacterium to be tested. The plate is incubated for 18-24 hrs at 37^{o}C. During this period, the antibacterial agent diffuses through the agar and may prevent the growth of organism. Effectiveness of susceptibility is proportional to the diameter of inhibition of zone around the disc. Organisms which grow up to the edge of the disc are resistant.

A) **Materials Required** Peptone, Sodium Chloride, Dil. Sodium Hydroxide, Dil. Sulphuric acid, Agar, Distilled water, pH paper, Conical flask, Culture tubes, Glass rod, Non-absorbant cotton, Autoclave- Micropipette, Petri dishes and Incubator.

B) Experimental Parts

Organisms used : *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebseilla pneumonia,*

Media used : Nutrient Agar.

Test used : Methanolic extract of *Acalypha indica* Linn., patch,
Standard : Ciprofloxacin, Nystatin

C) Preparation of Nutrient Agar

8.2 gm of agar powder was dissolved in 250 ml of water. The medium was steamed in boiler to precipitate any heat coagulable material. Then the medium was filtered. The filtrate was distributed in 5ml quantity in to culture tubes. The tubes were plugged with non-absorbent cotton. The medium in the tubes were sterilized by autoclave not less than 15 minutes at 15 pounds per sq. inch at 121^{o}C.

D) Preparation of Nutrient Broth

15.2 gm of nutrient broth agar was dissolved in 400 ml of water. To this required quantity of agar was added and heated in a beaker in order to dissolved agar. Then it was distributed in to culture tubes and plugged with cotton and sterilized in autoclave.

E) Inoculation of Culture Media

From nutrient broth solution, a quantity of 10 ml was taken in three test tubes inoculation needle or loop was heated in to red condition in the hot zone of Bunsen burner. The needle is allowed to cool or its own by holding it in the zone of sterility of Bunsen flame. The tube containing specimen culture grasped in the left hand probably in the horizontal or inclined position but not vertical. Mouth of the culture tube was passed through the Bunsen flame to kill any organisms that may fall in to the culture. Inoculation needle was inserted in to the specimen, culture tube containing actively

growing bacteria. The growth on the surface of agar was gently touched with the tip of the needle. The inoculation of charged needle was dripped in to the test tubes containing nutrient broth. The test tubes were kept aside for 24 hrs at 37^{o}c for 24 hours.

F) Preparation of Paper Disc

By using standard punching machine what man filter paper was cut and standard paper of 6.0 mm diameter was prepared. The paper discs were sterilized in a hot air oven at 160^{o}C for 1hour. The paper discs were then impregnated with the test solution.

6.16.2. Anti-Fungal Activity[53,54,]

Principle

Discs impregnated with known concentration of antibiotics discs are placed on Modified Sabouraud's Glucose agar plate that has been inoculated (or) seeded uniformly over the entire plate with a culture of the fungi to be tested. The plate is incubated for 3days 18-24 hrs at 37^{o}C. During this period, the antifungal agent diffuses through the agar and may prevent the growth of organism. Effectiveness of susceptibility is proportional to the diameter of inhibition of zone around the disc. Organisms which grow up to the edge of the disc are resistant.

A) Materials Required

Glucose, Peptone, yeast extract, Glycerin mono stearate, Olive oil, Tween 80, Chloramphenicol, Agar, Distilled water, pH paper, Conical flask, Culture tubes, Glass rod, Non-absorbant cotton, Autoclave, Micropipette Petri dishes and Incubator.[75]

B) Experimental Parts

Organisms used : *Candida albicans, Aspergillus niger.*

Media used : Modified Sabouraud's Glucose Agar medium

Test used : Methanolic extract of *Acalypha indica* Linn. patch,

Standard : Ciprofloxacin, Nystatin

7. RESULTS AND DISCUSSIONS

7.1. Absorption maxima (λ max) of Methanolic Extract of *Acalypha indica* Linn.

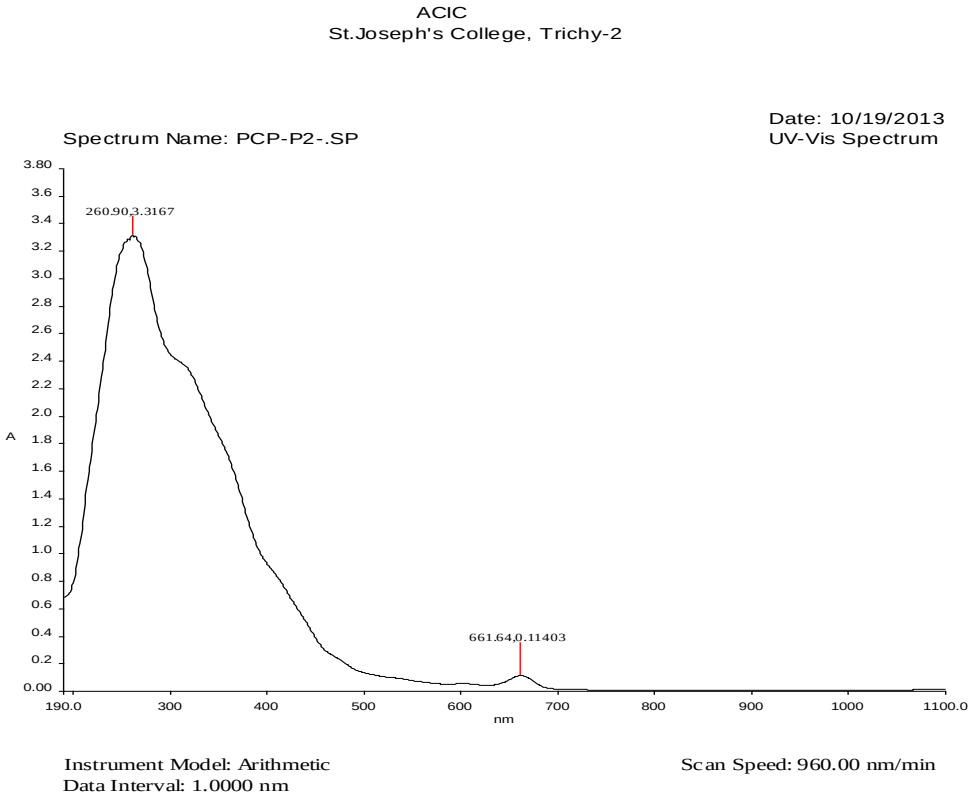


Fig No.17: Absorption maxima (λ max) of Methanolic Extract of *Acalypha indica* Linn.

From the sharp peak observed at 260 nm, further measurements were taken at 260 nm.

7.2. Standard curve of Methanolic Extract of *Acalypha indica* Linn. :

Table No:. 6: Standard Cure of Extract of *Acalypha indica* Linn.

Concentration (μg/ml)	Absorbance
	Average±SD
0	0.000± 0.000
5	0.091± 0.001
10	0.182± 0.001
15	0.273± 0.001
20	0.363± 0.001
25	0.459± 0.008

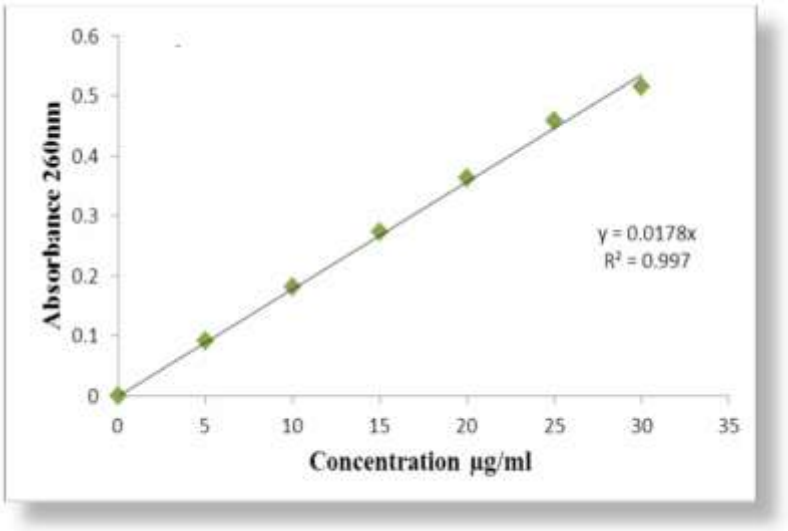


Fig No.18: Standard Cure of Methanolic Extract of *Acalypha indica* Linn.

7.3. Physical appearance

Colour : Green

Taste : Bitter taste

Solubility : Freely soluble in Methanol

7.4. Extraction of Leaves of *Acalypha indica* Linn.

MEA was carried out as per the procedure described in 6.3 and the yield of the extract was 6.5%w/v.

7.5. Phytochemical Studies

The phytochemical studies revealed that the presence of alkaloids, saponins, tannins, phenolic compounds, flavonoids and sterol.

Table No.7: Phytochemical constituents

S.NO	Chemical constituents	Alcoholic extract
1.	Alkaloids	+
2.	Saponins	+
3.	Tannins	+
4.	Phenolic compounds	+
5.	Flavonoids	+
6.	Steroids	+
7.	Glycosides	+
8.	Amino acids	+
9.	Reducing sugar	-
10.	Terpenoids	-

(+) Presence of constituents

(-) Absence of constituents

7.6. Hygroscopic Nature

Table No: 8 Hygroscopic Nature determination

At Room Temperature	**75%RH at 40^0C**
Sample No-1	Sample No-1
Weight gain observed-Nil	Weight gain observed-Nil

From the results, it is observed that the prepared Transdermal patch is non hygroscopic in nature

7.7. Compatibility study

FTIR studies

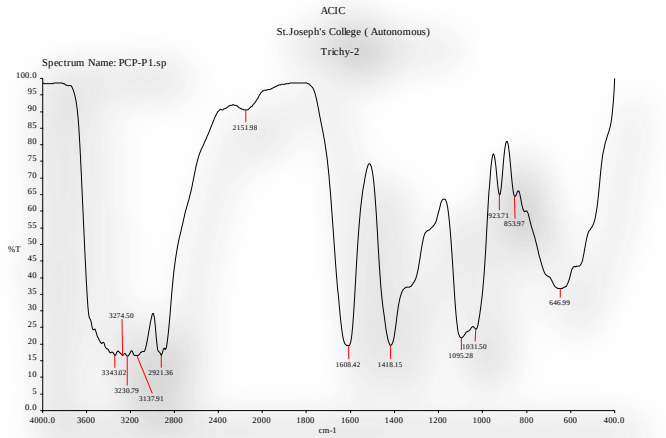


Fig No. 19: FTIR Spectrum of Methanolic Extract of *Acalypha indica* Linn.

Table No. 9: FTIR Interpretation of Methanolic Extract of *Acalypha indica* Linn.

Wave number (cm^{-1})	Functional Group
3782.15	OH Stretching
3346.50	OH Stretching
3055.53	C-H Stretching
2923.11	C-H Stretching
1639.23	C-H Stretching

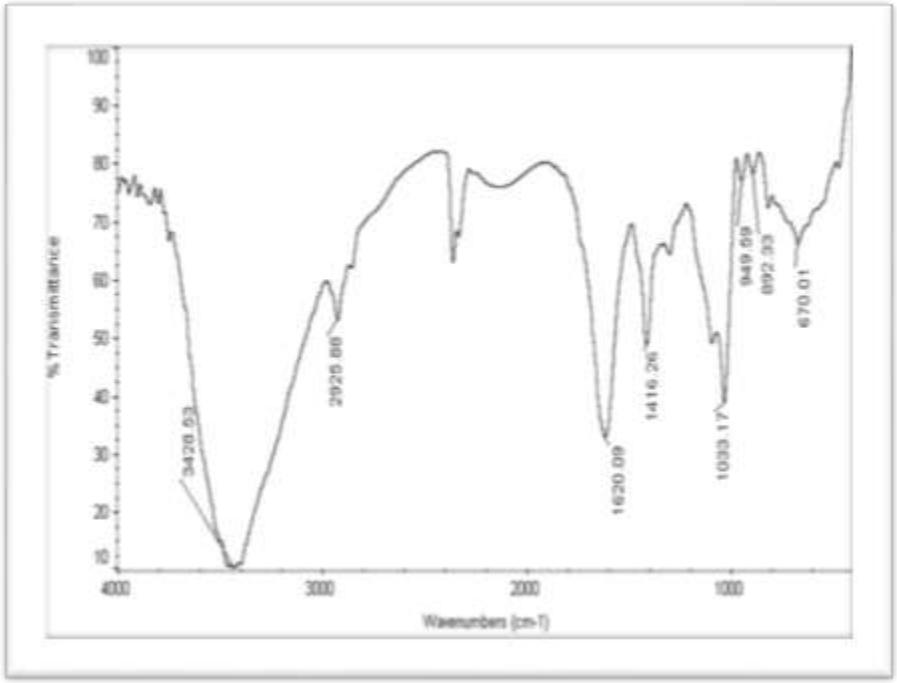


Fig No. 20: FTIR spectrum of Pectin

Table No.10: FTIR Interpretation of Pectin

Wave number (cm-1)	Functional groups
3442.09	C=O stretching
1931.41	C-C stretching
1742.41	C-C stretching
1232.88	OH bending
914.15	C-O Stretching
769.94	CH Rocking

Fig No. 21: FTIR of Spectrum Sodium alginate

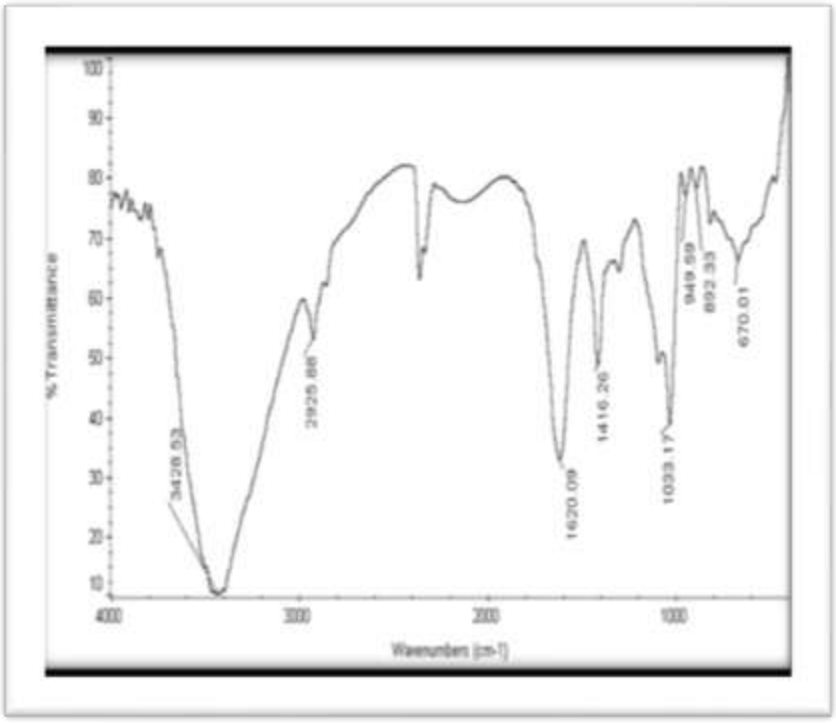


Table No.11: FTIR Interpretation of Sodium alginate

Wave number (cm^{-1})	Functional group
3428.53	O-H Stretching
2925.88	C-H Stretching
1620.09	C=O Strtching
1416.26	C-O Stretching
1033.17	C-C Stretching
949.59-670.01	C-H (Out plane bending)

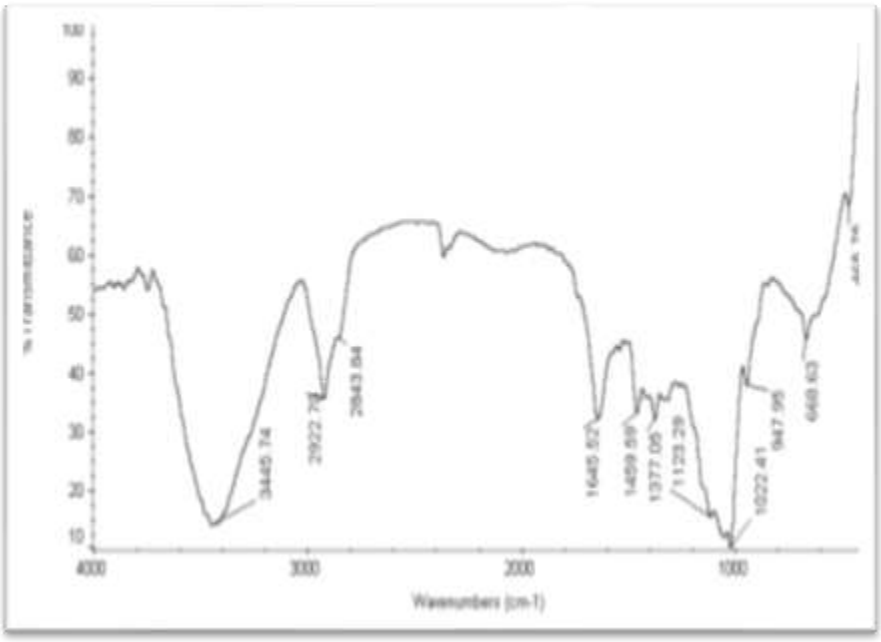


Fig No. 22: FTIR Spectrum of HPMC

Table No. 12: FTIR Interpretation of HPMC

S.No.	Wavenumber (cm^{-1})	Functional groups
1	3445.74	O-H stretching
2	2922.70	C-H stretching
3	1645.52,1459.59	C-C multiple bond stretching
4	1377.05	O-H stretching

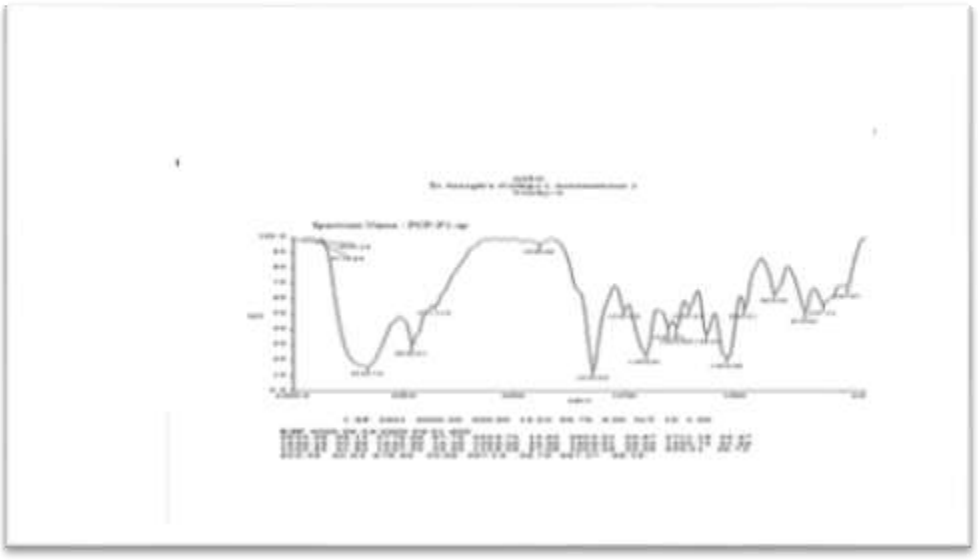


Fig No. 23: FTIR Spectrum of Pectin Formulation(P2)

Table No.13 :FTIRInterpretation of Pectin formulation

Wave number (Cm^{-1})	Functional groups
1644.88	C=C stretching
1412.89	O-H bending
1045.91	C-O stretching
1115.29	C-O stretching
1045.91	C-O stretching

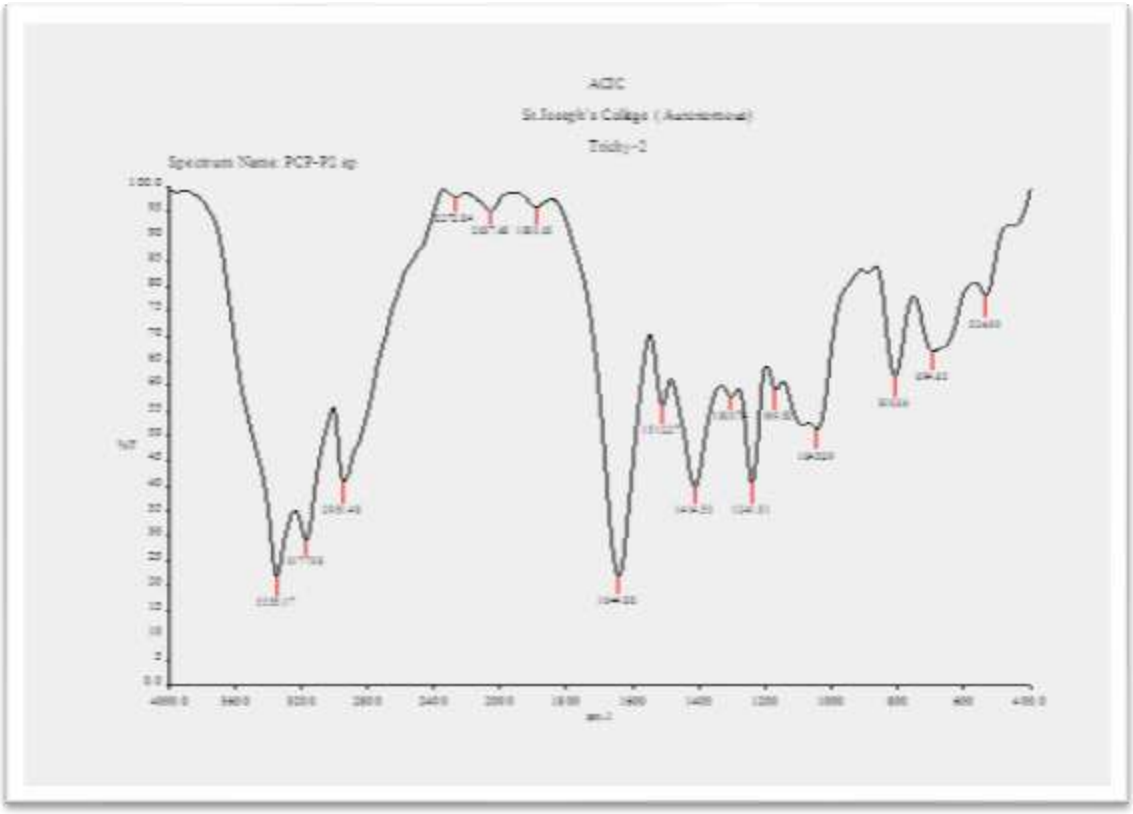


Fig No. 24: FTIR Spectrum of HPMC formulation(H4)

Table No.14: FTIR Interpretaion of HPMC formulation

Wave number (Cm^{-1})	Functional groups
3339.75	O-H stretching
1412.89	O-H bending
1045.91	C-O stretching
1115.29	C-O stretching
1045.91	C-O stretching
954.38	C-H (Outplane bending)
675.03	C-H (Outplane bending)

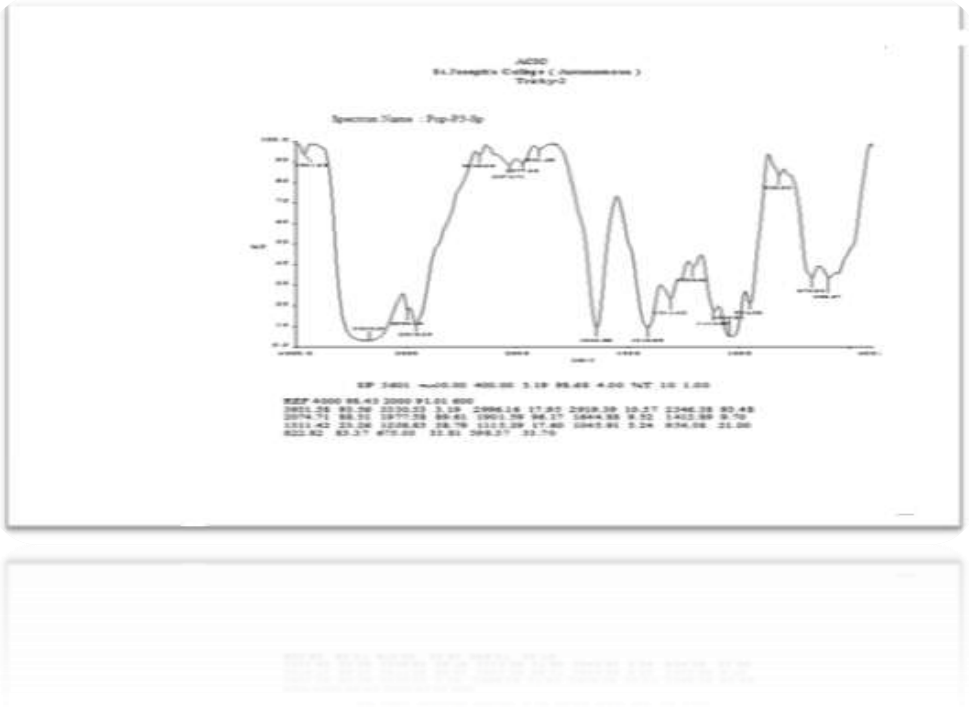


Fig No.25: FTIR Spectrum of Sodium Alginate Formulation(s7)

Table No.15: FTIR Interpretation of Sodium alginate formulation

Wave number (Cm^{-1})	Functional groups
3776.66	O-H stretching
3324.72	O-H stretching
1643.60	C=C stretching
1265.8	C-H (in plane bending)
679.94	C-H (Out plane bending)

The FTIR graphs of drug ,excipients and formulations results showed that there is no extra peak (or) broadening of peaks were observed and thus it indicates that there is no incompatibility between drug and excipients.

7.8. Physico chemical evaluation of Methanolic Extract of *Acalypha indica* Linn. Transdermal patch

Table No. 16:Physico chemical evaluation of Methaolic Extract of *Acalypha indica Linn.*Transdermal patch

Formulation code		Uniformity of weight (g)	Thickness (mm)	Drug content (%)	Folding Endurance(no's)	Moisture Uptake (%)	Moisture Content (%)	Surface pH	Percent Elongation (% mm)	Tensile Strength (Kg/mm^2)
Pectin (P)	P1	0.23±0.73	0.17±0.52	95.47±0.05	260±0.06	2.06±0.54	0.572±0.60	6.4±0.05	66±0.52	3.410±0.05
	P2	0.37±0.1	0.23±0.47	95.82±0.65	270±0.57	2.14±0.08	1.925±0.4	7.2±0.10	85±043	5.400±0.65
	P3	0.41±0.45	0.29±0.61	95.70±0.68	240±0.82	1.97±1.03	1.624±0.05	6.2±0.72	78±1.21	6.461±0.83
HPMC(H)	H4	0.37±0.01	0.35±0.15	96.24±0.65	285±0.57	3.57±0.07	3.271±0.4	7.4±0.11	130±0.21	7.660±0.21
	H5	0.26±0.20	0.32±0.83	96.07±0.29	259±0.41	2.76±0.67	2.967±0.03	7.2±0.43	125±0.72	6.461±0.46
	H6	0.17±0.25	0.24±0.59	95.93±0.81	245±0.64	2.95±0.34	3.431±0.62	7.2±0.9	118±0.92	5.410±0.72
Sodium alginate (S)	S7	0.28±0.61	0.11±0.51	96.00±1.04	237±0.38	0.96±0.41	1.481±0.45	7±0.65	98±0.06	3.800±0.20
	S8	0.25±0.2	0.18±0.32	96.01±0.47	267±1.0	1.88±0.19	0.192±0.08	6.6±0.30	113±0.89	4.660±0.89
	S9	0.31±1.26	0.20±0.45	95.76±0.70	245±0.92	1.89±0.02	1.923±1.01	6.6±0.27	86±0.79	6.410±1.63

Mean±S.D: n=3

The nine (P1,P2,P3,H4,H5,H6,S7,S8,S9) batches of drug loaded films with different ratios of different polymers were subjected to various physicochemical evaluations.

Based on thickness, uniformity of weight, folding endurance, percentage moisture uptake and tensile strength,the formulations P2, H4, and S7 were selected for further studies.

7.9. Optimized formula of *Acalypha indica Linn.* Transdermal patch

Table No.17:Optimized formula of *Acalypha indica* Linn. Transdermal patch

S.NO	Ingredients	P (Pectin)	H (HPMC)	S (Sodium alginate)
1	Extract (mg) *Acalypha indica Linn.*.	10	10	10
2	Polymer (mg)	10	20	40
4	DMSO/SLS(ml)	0.3	0.3	0.3
5	Glycerin (ml)	0.3	0.3	0.3
3	Water (ml)	q.s	q.s	q.s

7.10. Selected Formulations

Fig No. 26: Transdermal Patch of (P_2) Pectin formulation

Fig No .27: Transdermal Patch of (H_4)HPMC Formulation

Fig No. 28: Transdermal Patch of (S_7) Sodium alginate formulation

7.11. Physicochemical Evaluation of Selected formulations (P2,H4 and S7)

Table No. 18: Uniformity of weight

S.No	Formulation code	Weight(g)
1	Pectin	0.19±0.1
2	HPMC	0.21±0.01
3	Sodium alginate	0.15±0.2

Mean ± S.D: n= 3

Table No. 19: Thickness of the patch

S.No	Formulation code	Thickness (mm)
1	Pectin	0.23±0.47
2	HPMC	0.35±0.15
3	Sodium alginate	0.18±0.32

Mean± S.D:n=3

Table No. 20: Determination of Drug content

S.No	Formulation code	% Drug content
1	Pectin	95.82±1.21
2	HPMC	96.24±0.65
3	Sodium alginate	96.01±1.05

Mean ± S.D: n=3

Table No. 21: Folding Endurance

S.No	Formulation code	Folding Endurance (No's)
1	Pectin	255±1.5
2	HPMC	265±0.57
3	Sodium alginate	260±1.0

Mean ± S.D: n =3

Table No. 22: Percentage Moisture Uptake

S.No	Formulation code	% Moisture uptake
1	Pectin	2.14±0.08
2	HPMC	1.57±0.07
3	Sodium alginate	2.88±0.19

Mean ± S.D: n=3

Table No. 23: Percentage Moisture Content

S.No	Formulation code	% Moisture content
1	Pectin	1.92±0.6
2	HPMC	1.67±0.4
3	Sodium alginate	1.95±0.2

Mean ± S.D: n=3

Table No. 24: Surface pH

S.No	Formulation code	Surface pH of patches
1	Pectin	7.2±0.10
2	HPMC	7.4±0.11
3	Sodium alginate	6.6±0.30

Mean ± S.D: n=3

Table No. 25: Percent Elongation

S.No	Formulation code	% Elongation (%mm)
1	Pectin	92±0.43
2	HPMC	97±0.26
3	Sodium alginate	89±1.04

Mean ± S.D: n=3

Table No.26: Tensile Strength

S.No	Formulation code	Tensile strength (kg/mm^2)
1	Pectin	5.300±0.65
2	HPMC	6.660±0.25
3	Sodium alginate	4.245±0.78

Mean ± S.D: n=3

Table No. 27: *In-vitro drug* release profile of P_2

S.No	Time (min)	% Drug release
1	0	0
2	5	0.51± 0.23
3	10	0.80 ± 0.45
4	15	1.54 ± 0.44
5	30	2.20 ± 0.76
6	45	5.45 ± 1.78
7	60	10.00 ± 0.59
8	120	19.38± 1.23
9	180	25.48 ± 0.61
10	240	34.56 ±1.24
11	300	38.73 ± 1.90
12	360	44.54±1.54
13	420	49.00± 0.23
14	480	57.64± 1.34
15	540	62.28 ± 0.91
16	600	68.24± 0.72
17	660	77.36± 0.61
18	720	85.24 ± 0.72

Mean±S.D:n=3

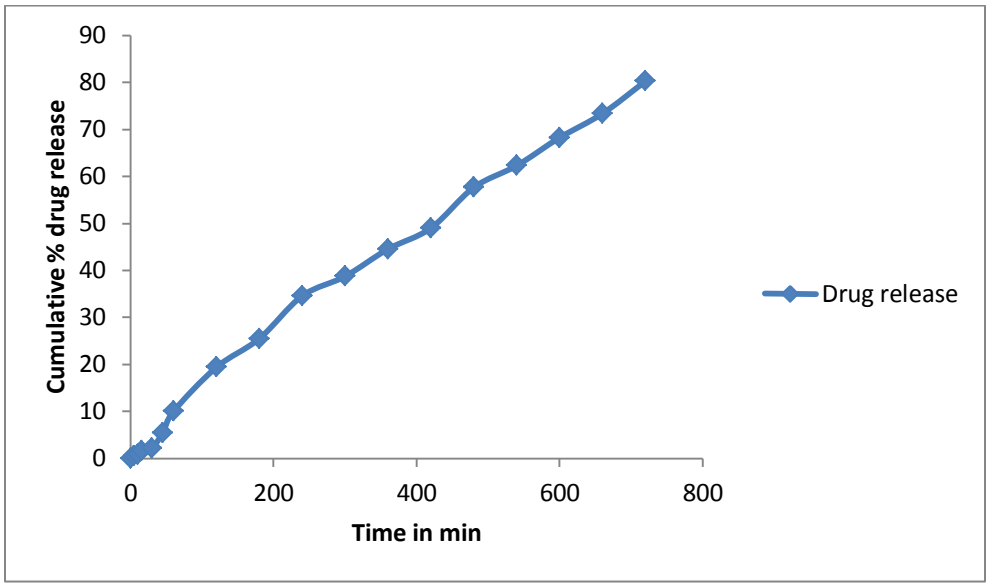


Fig No. 29: *In- vitro* drug release profile of P_2

Table No. 28 *: In- vitro* drug release profile of H_4

S.No	Time (min)	% Drug release
1	0	0
2	5	0.33 ± 0.07
3	10	0.74± 0.64
4	15	1.00 ± 0.27
5	30	2.14 ± 0.05
6	45	4.23 ± 0.73
7	60	8.00 ± 0.73
8	120	17.31 ± 1.2
9	180	23.45 ± 0.05
10	240	31.23 ± 0.59
11	300	38.42 ± 0.01
12	360	45.58 ± 0.48

13	420	51.25 ± 0.12
14	480	57.25 ± 0.73
15	540	68.46 ± 0.89
16	600	73.46 ± 0.41
17	660	75.34±0.23
18	720	70.56±0.41

Mean S.D:n=3

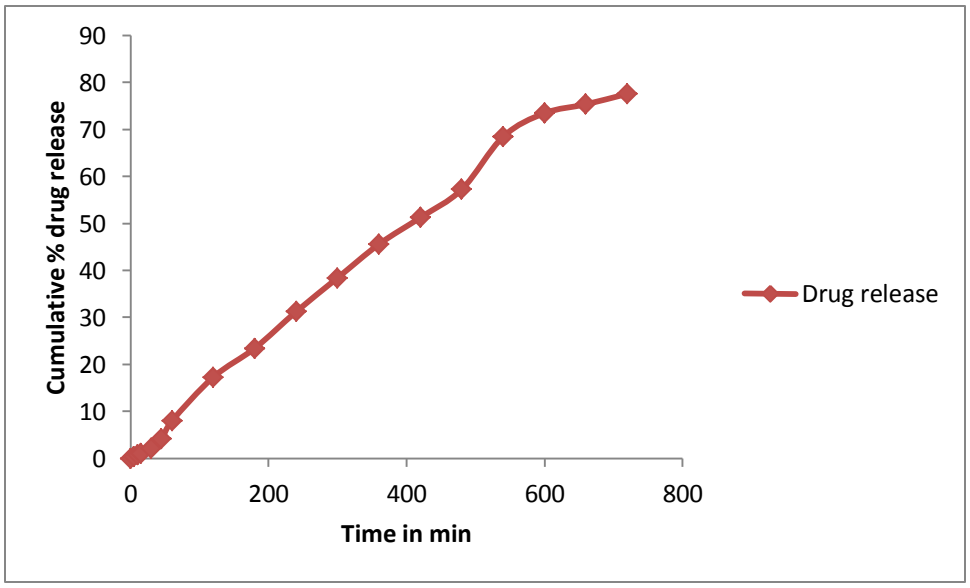


Fig No. 30: *In- vitro* drug release profile of H_4

Table No. 29 *: In- vitro* drug release profile of S_7

S.No	Time (min)	% Drug release
1	0	0
2	5	0.61 ± 0.64
3	10	1.45 ± 0.52
4	15	2.01 ± 0.03
5	30	4.27 ± 0.09
6	45	6.98 ± 0.62
7	60	11.53 ± 0.17
8	120	20.42 ± 0.06
9	180	26.56 ± 0.06
10	240	34.28 ± 0.41
11	300	41.58 ± 0.24
12	360	45.95 ± 0.41
13	420	52.00 ± 0.06
14	480	58.74 ± 0.92
15	540	65.48 ± 0.68
16	600	71.41 ± 0.46
17	660	76.76 ± 0.86
18	720	84.41 ± 0.53

Mean± S.D n=3

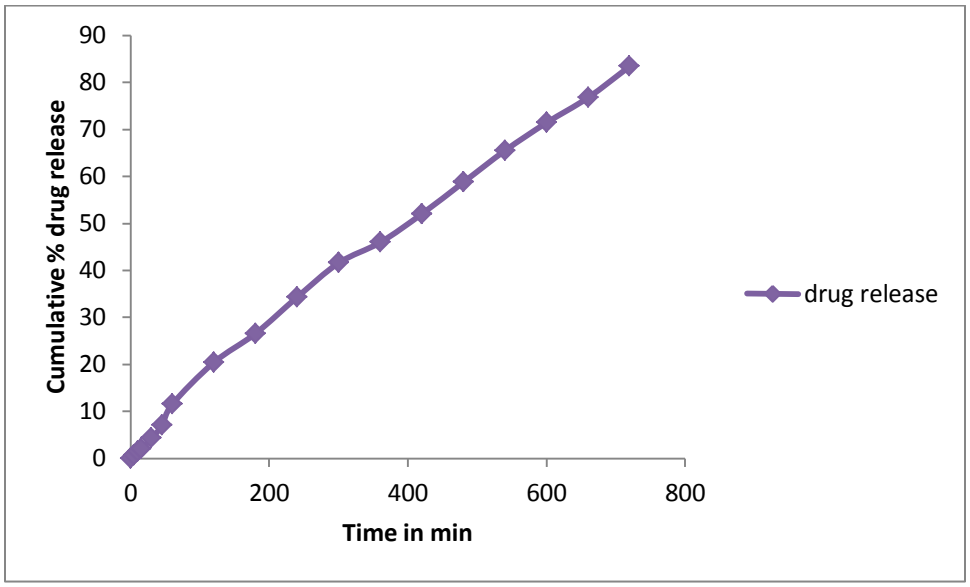


Fig No. 31: *In -vitro* drug release profile of S_7

Table No.30 : Comparative *in- vitro* drug release profile

S.No	Pectin	HPMC	Sodium alginate
1	0	0	0
2	0.51± 0.23	0.33 ± 0.07	0.61 ± 0.64
3	0.80 ± 0.45	0.74± 0.64	1.45 ± 0.52
4	1.54 ± 0.44	1.00 ± 0.27	2.01 ± 0.03
5	2.20 ± 0.76	2.14 ± 0.05	4.27 ± 0.09
6	5.45 ± 1.78	4.23 ± 0.73	6.98 ± 0.62
7	10.00 ± 0.59	8.00 ± 0.73	11.53 ± 0.17
8	19.38± 1.23	17.31 ± 1.2	20.42 ± 0.06
9	25.48 ± 0.61	23.45 ± 0.05	26.56 ± 0.06
10	34.56 ±1.24	31.23 ± 0.59	34.28 ± 0.41
11	38.73 ± 1.90	38.42 ± 0.01	41.58 ± 0.24

12	44.54±1.54	45.58 ± 0.48	45.95 ± 0.41
13	49.00± 0.23	51.25 ± 0.12	52.00 ± 0.06
14	57.64± 1.34	65.26 ± 0.73	58.74 ± 0.92
15	62.28 ± 0.91	68.46 ± 0.89	65.48 ± 0.68
16	68.24± 0.72	73.46 ± 0.41	71.41 ± 0.46
17	77.36± 0.61	75.34±0.23	76.76 ± 0.86
18	80.24 ± 0.72	70.56±0.41	83.41 ± 0.53

Mean ±S.D n=3

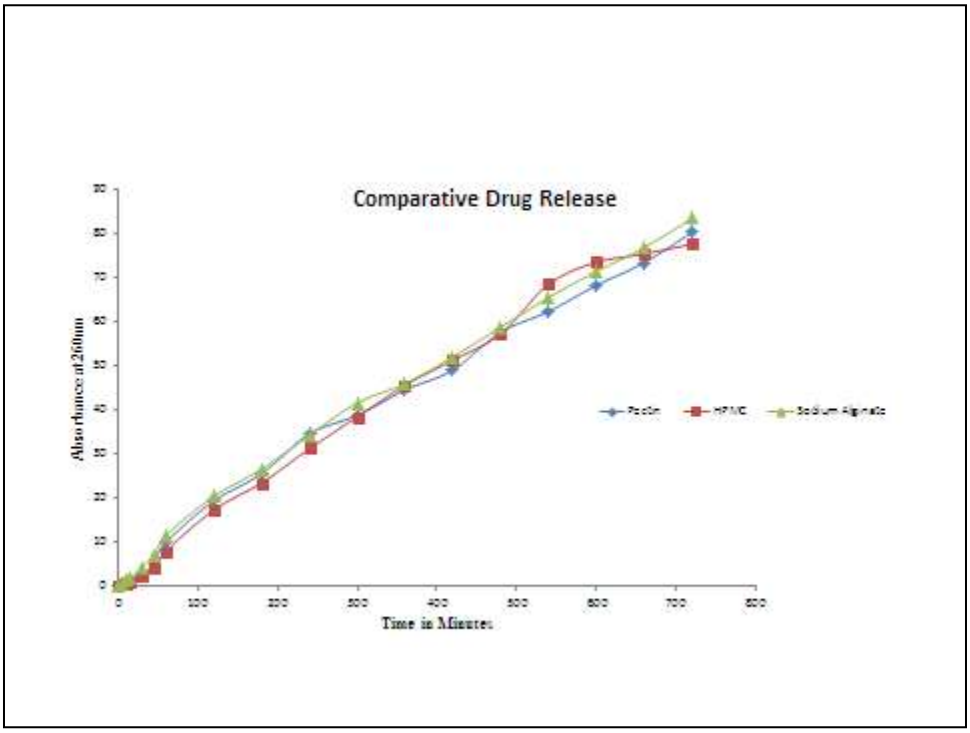


Fig No.3 2: Comparative *in -vitro* drug release profile

The selected 3 batches of formulations Pectin (P2), Sodium alginate (S7) and HPMC (H4) were subjected *ex-vivo* diffusion studies. Formulation H4 showed sustained release in a controlled manner upto 12 hrs. So this has been selected for *ex-vivo* and stability studies.

Fig No. 33 *: Ex- vivo* diffusion study (H4)

Fig No. 34 :Goat abdomen skin tied on open ended cylinder

Table No: 31. *Ex- vivo* Transdermal Permeation of H_4

S.No	Time in min	% Drug release
1	0	0
2	5	0.30±0.15
3	10	0.43±0.53
4	15	0.70±0.71
5	30	1.35±0.62
6	45	2.23±0.45
7	60	3.12±0.47
8	120	5.45±0.16
9	180	11.28±0.32
10	240	20.45±0.06
11	300	27.70±0.83
12	360	35.20±0.71
13	420	40.56±0.71
14	480	50.24±0.63
15	540	53.26±0.68
16	600	62.43±0.24
17	660	68.73±0.63
18	720	70.24±0.58
19	780	65.24±0.25

Mean ± S.D: n= 3

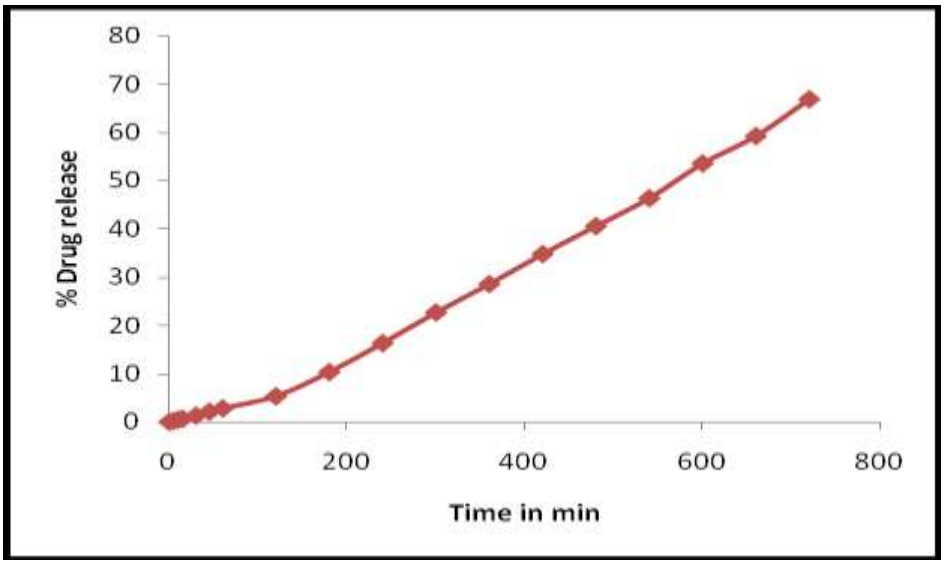


Fig No. 35: *Ex- vivo* Transdermal Permeation of H_4

7.12. Release Kinetics

Table No.32 *: In-vitro* values are subjected to various kinetics equations

Formulation Code	Correlation Coefficient (r^2)				
	Zero order	**First order**	**Higuchi**	**Korsmeyrpeppas**	**'n'-Release Exponent**
P2	0.991	0.763	0.972	0.927	0.86
H4	0.991	0.797	0.963	0.984	0.83
S7	0.992	0.756	0.973	0.995	0.87
Ex- vivo(H4)	0.990	0.867	0.930	0.995	0.86

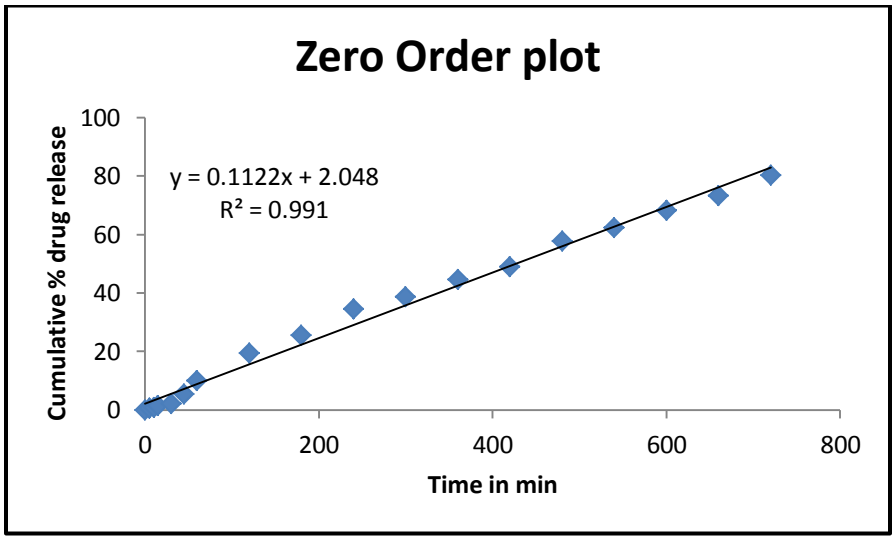


Fig No. 36: Release kinetics of P2

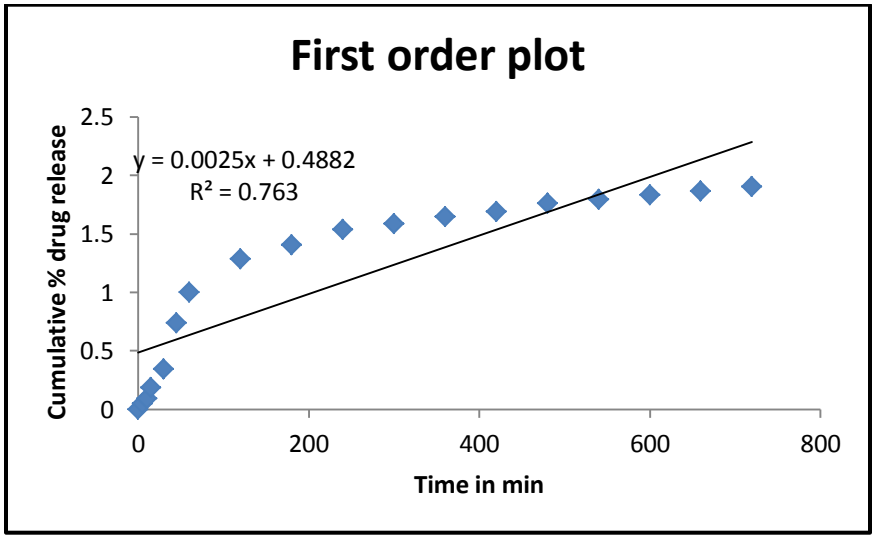
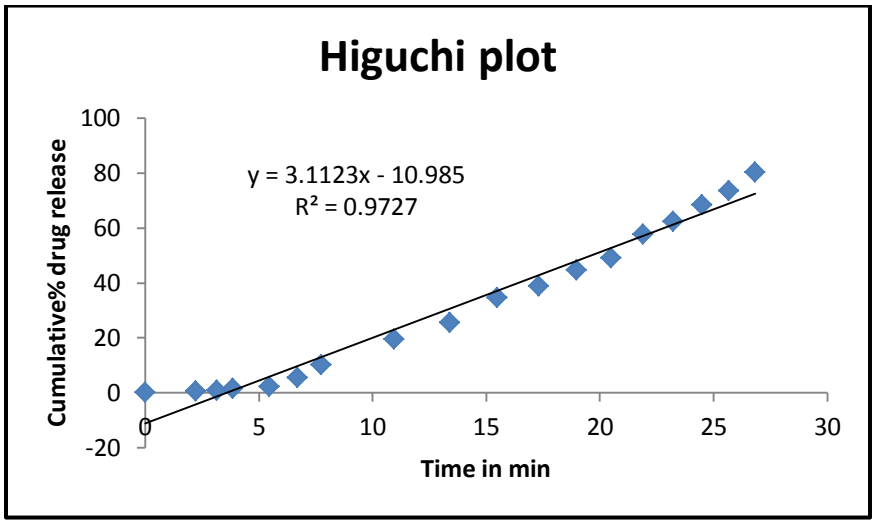


Fig No. 37



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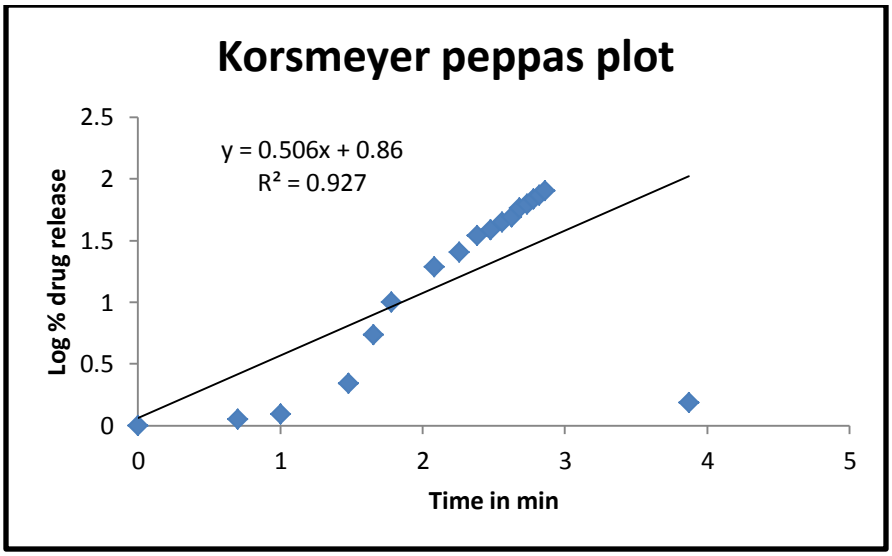
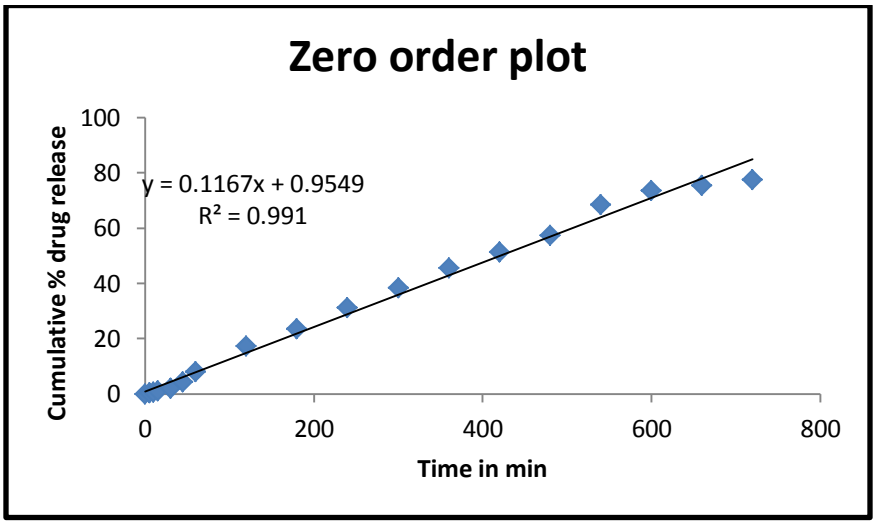


Fig No. 39

Release kinetics of H_4



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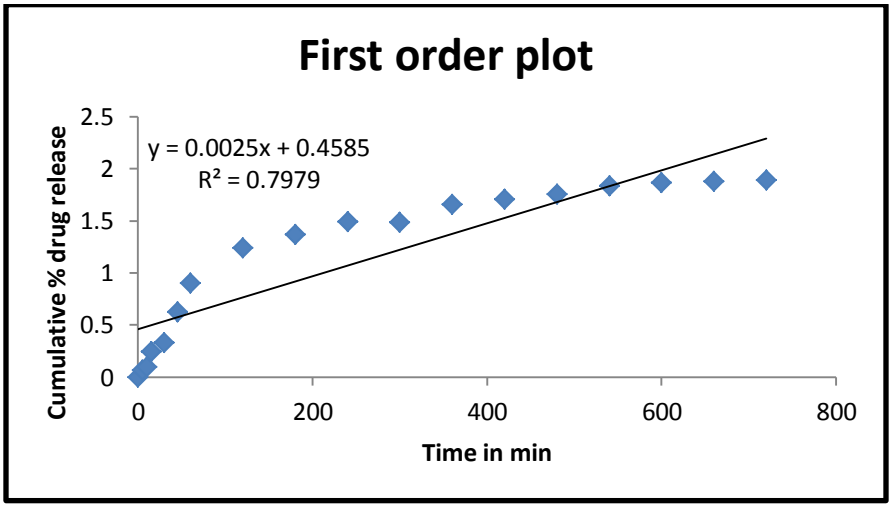

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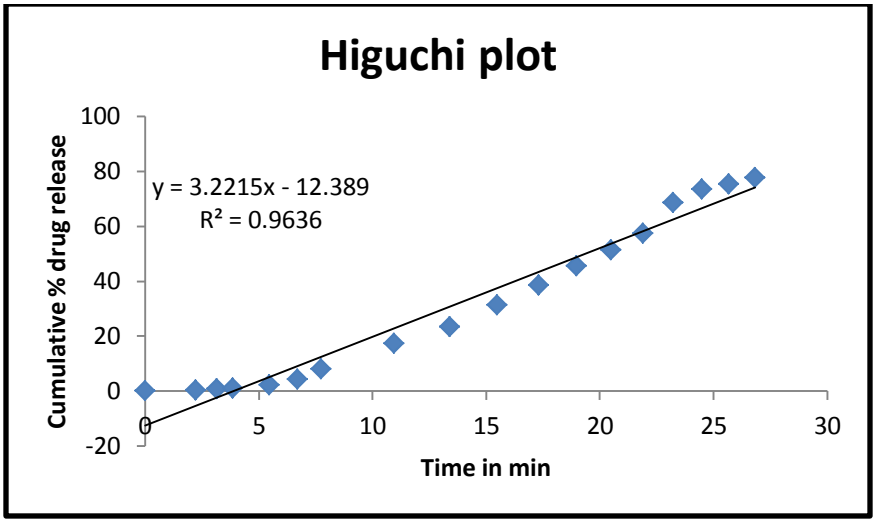

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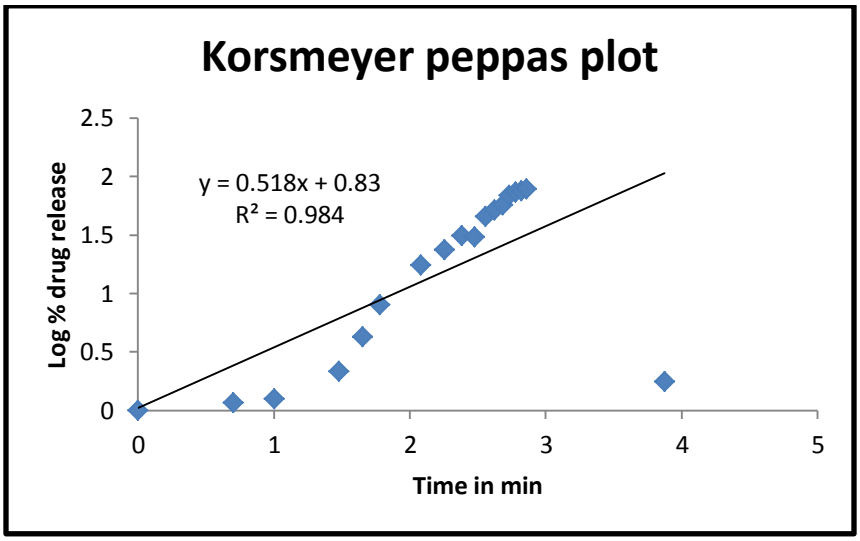
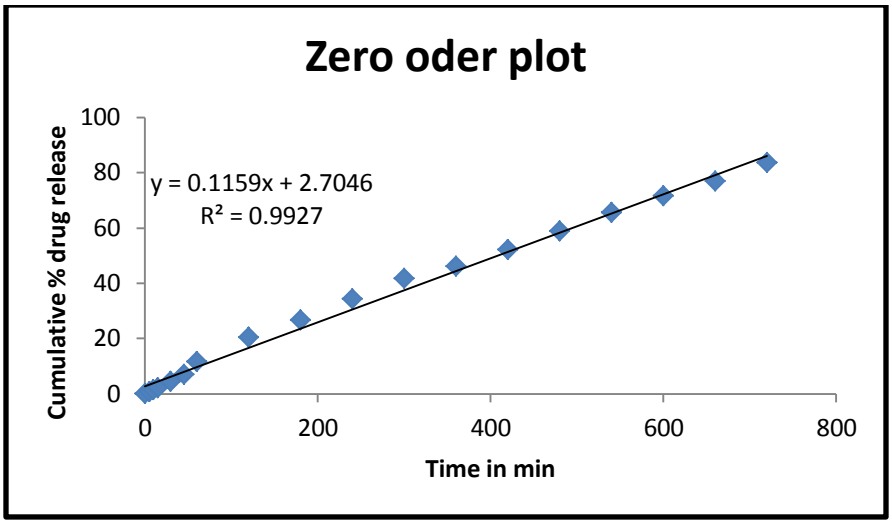


Fig No. 43

Release kinetics of S7



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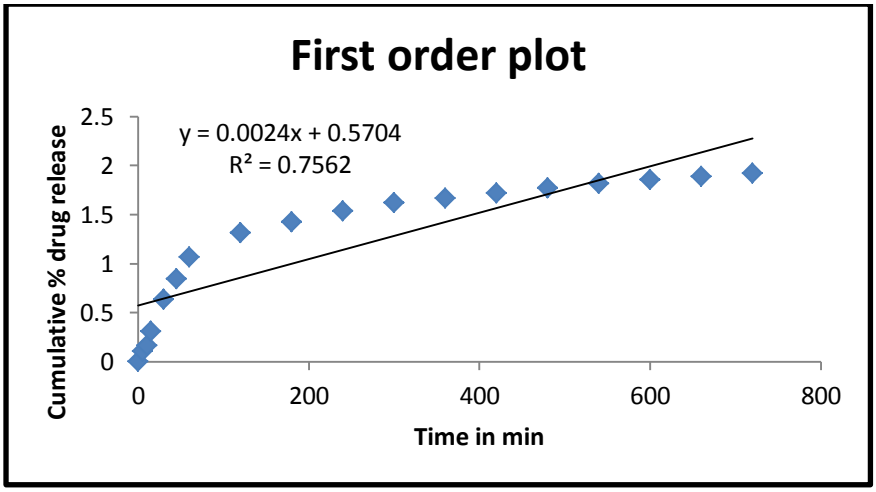
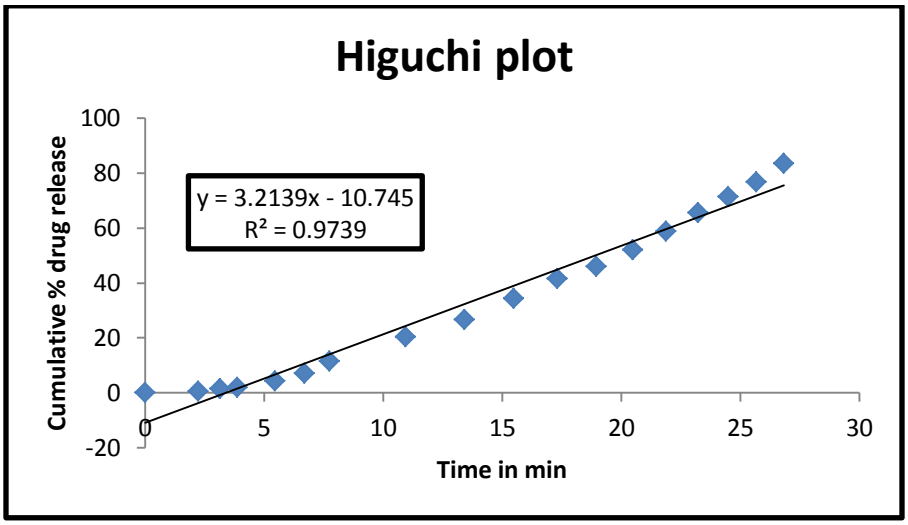


FigNo.45



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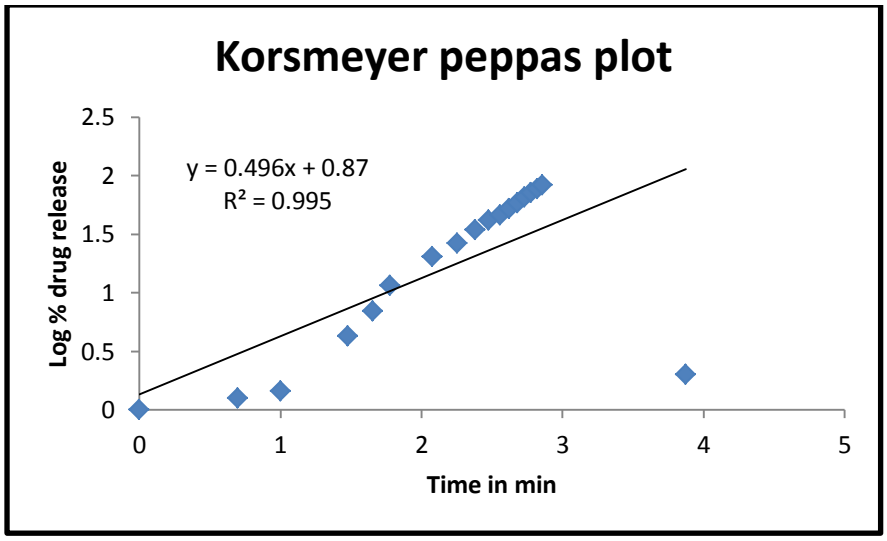

Fig No. 47

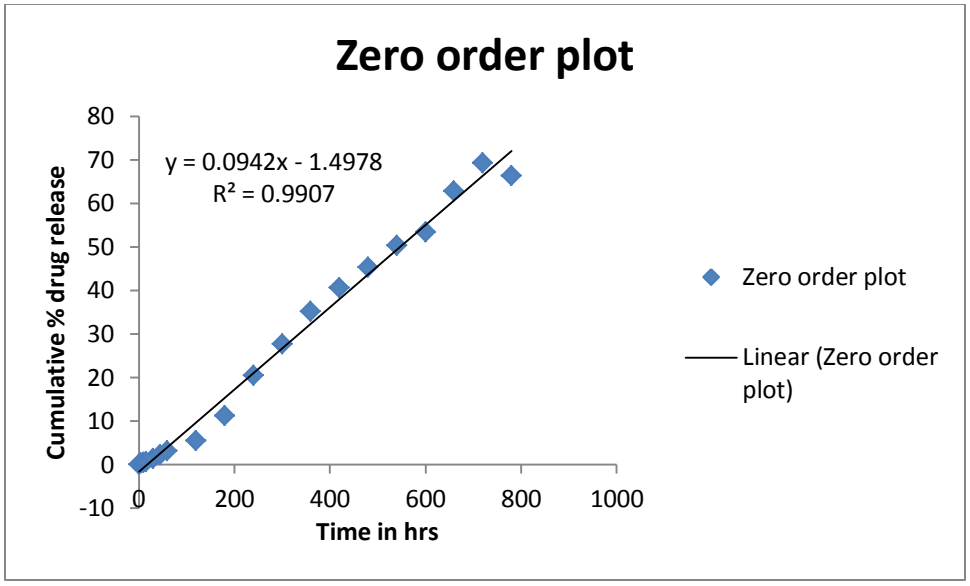

Fig No. 48: Ex-vivo Release of H4

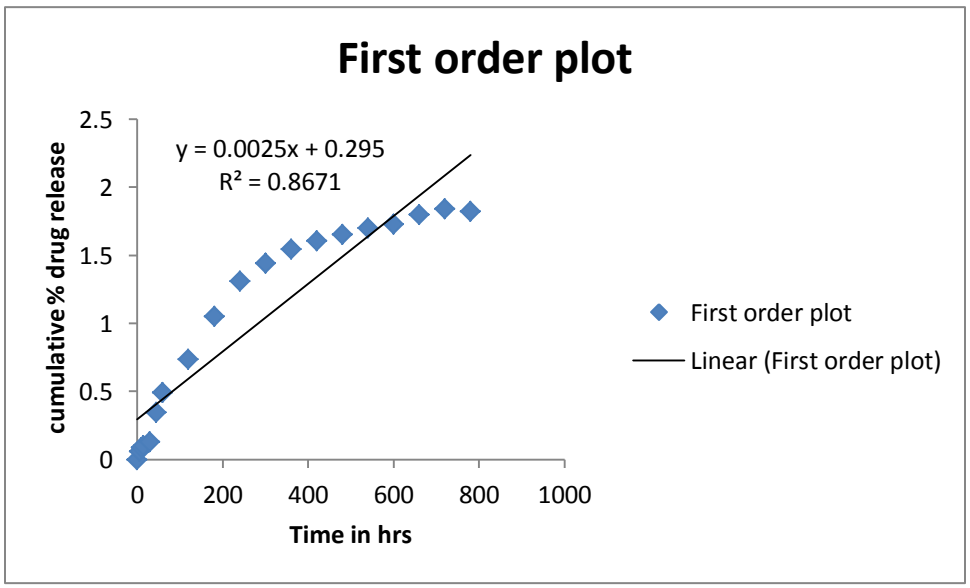

Fig No. 49

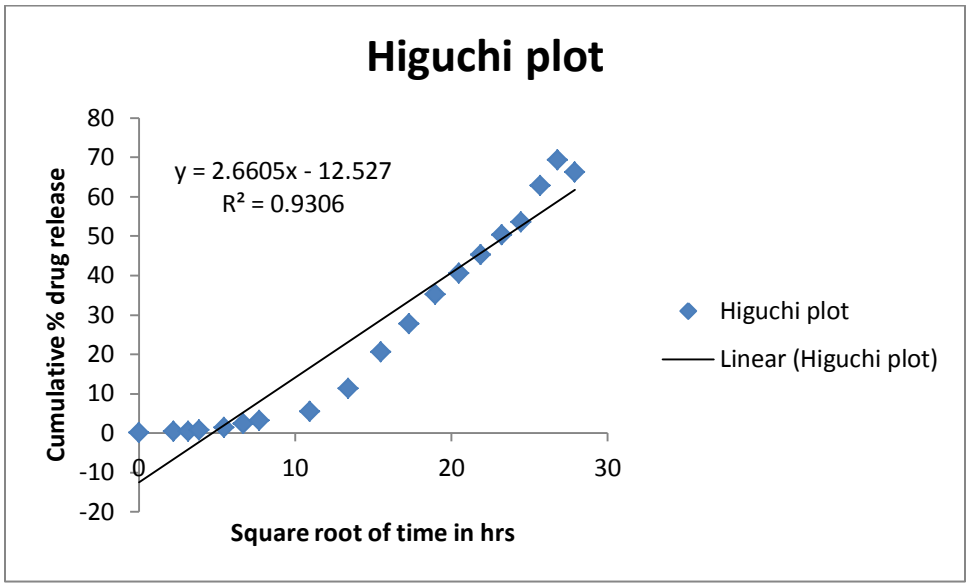

Fig No. 50

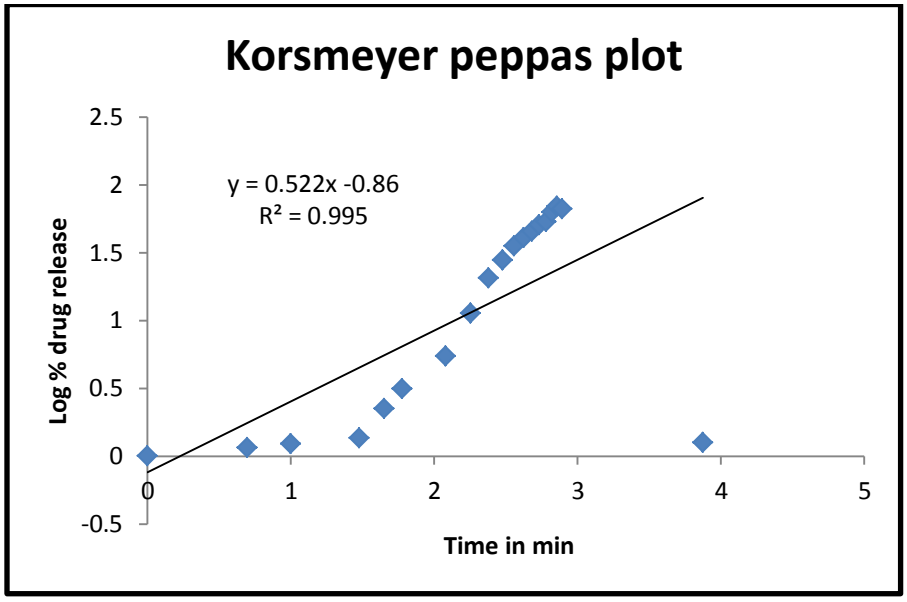

Fig No.51

The release kinetics showed that the H4 formulation followed Zero order and non-fickian diffusion.

7.13. Stability study of Methanolic Extract of *Acalypha indica* Linn.Transdermal Patch

Table No: 33

Parameter	**Room temperature**	**40±2^{0}C& RH 70±5%**
Visual Appearance	Transparent	Transparent
Initial	No change	No change
At the end of 1st month	No change	No change
At the end of 2nd month	No change	No change
At the end of 3rd month	No change	No change
Colour	Dull white	Dull white
Initial	No change	No change
At the end of 1st month	No change	No change
At the end of 2nd month	No change	No change
At the end of 3rd month	No change	No change
Texture	Smooth	Smooth
Initial	No change	No change
At the end of 1st month	No change	No change
At the end of 2nd month	No change	No change
At the end of 3rd month	No change	No change
Drug content	No change	No change
Initial	**96.24%**	**96.24%**
At the end of 1st month	96.22%	96.14%
At the end of 2nd month	95.95%	95.81%
At the end of 3rd month	95.21%	95.06%

The stability studies of H4 formulation of Methanolic Extract of *Acalypha indica* Linn.Transdermal Patch was carried out for 3 months as per the procedure described in the Chapter.6. During this period, the formulations were stable and showed no significant changes in Visual appearance, Colour, Texture and Drug content.

7.14. Screening of Antimicrobial activity of *Acalypha indica Linn.*

FUNGI

Fig No. 52. ***Aspergillusniger***

Fig No. 53. ***Candida albicans***

GRAM POSITIVE BACTERIA

Fig No. 54.*Bacillus subtilis*

Fig No.55.*Staphylococcus aureus*

GRAM NEGATIVE BACTERIA

Fig No. 56.*E.coli*

FigNo. 57.*Pseudomonas aeruginosa*

Fig No. 58. *Klebsiella pneumonia*

Table No: 34

S.No	Name of the microorganism	Zone of inhibition in mm				
		Extract of *Acalypha indica* Linn.				
		A	B	C	D	E
1	*Staphylococcus aureus* (NCIM 2079)	11	10	24	-	34
2	*Bacillus subtilis* (NCIM 2063)	12	12	09	-	12
3	*Pseudomonas aeruginosa* (NCIM 2036)	15	12	22	-	23
4	*E. coli* (NCIM 2065)	15	12	32	-	42
5	*Klebsiella pneumonia* (NCIM 2098)	14	12	38	-	45
6	*Aspergilus niger* (NCIM 105)	15	15	24	-	32
7	*Candida albicans* (NCIM 3102)	15	10	15	-	26

A= high concentration; B=low concentration; C=patch; D= DMSO; E=standard (ciprofloxacin 5µg/ disc for bacteria; Nystatin 100 µg/ disc for fungi)

Observation

The anti- microbial activity for the given sample was carried out by disc diffusion technique (Indian pharmacopoeia 1996, vol II A-105). The test micro organism of *Staphylococcus aureus, E.coli, Pseudomonas aeruginosa* and *Bacillus subtilis, Klebsiella* and Fungus *Aspergilusniger, Candida albicans* were obtained from national chemical laboratory (NCL) pune and maintained by periodical sub culturing on nutrient agar and sabouraud dextrose agar medium for bacteria and fungi respectively. The effect produced by the sample was compared with the effect produced by the positive control (reference standard Ciprofloxacin 5µg/ disc for bacteria; Nystatin100µg/ disc for fungi).

For Bacteria

After 24hrs the plates are observed. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no bacterial growth around the hole. The Figures are shown in 54,55,56,57,58 and results are in Table No: 34.

For Fungi

After 72hrs the plates are observed. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no fungal growth around the hole. The figures are shown in 52, 53 and the results are shown in Table No: 34.

8.1. SUMMARY AND CONCLUSION

Nine patches (P1, P2, P3, H4, H5, H6, S7 S8 and S9) of Methanolic Extract of *Acalypha indica* Linn. Transdermal Patches were prepared by Solvent Casting Technique.

Various formulation parameters, polymer (Pectin, HPMC and Sodium alginate) ratios and permeation enhancers (SLS and DMSO) were optimized to get thin, transparent, smooth, stable and high permeable Transdermal Patch.

The FTIR graphs of drug, excipients and formulations showed that there is no extra peak (or) broadening of peaks were observed and thus it indicates that there is no incompatibility between drug and excipients.

From the optimization, best 3 formulations of Pectin, Sodium alginate, HPMC (P2, H4 and S7) were selected based on folding endurance and optimal tensile strength.

When the amount of polymer was less than 0.4gm or greater than 0.4gm the folding endurance of the patch was affected. If the quantity less than 0.4gm handling of the patch was very difficult and if the quantity was greater than 0.4gm, the patch become thick or break with insignificant tensile strength.

0.3 ml of DMSO was selected as permeation enhancer to produce flexible patch without having major influence on their release property.

0.3 ml of glycerin was added as a plasticizer and to produce a flexible patch without having major influence on their release property. If the amount exceeds, the patch loses its flexibility and become stiff, hence the above two variables were controlled to form a good patch.

The plasticizer diffuses through the patch and softens the polymer particles. This softening promotes latex coalescence and patch formation.

All batches were evaluated for % Moisture uptake, % Moisture content, Thickness, Folding endurance, % Drug content, Percent elongation, Tensile strength.

Among these, the formulation H4 showed maximum % Moisture uptake (3.57), % Moisture content (3.27), Thickness (0.35mm), folding endurance (265), % Drug content (96.24), Percent elongation (97), Tensile strength (7.66 Kg mm^2).

No significant difference in drug content was observed between the patches among the three formulations.

The *in-vitro* release profile of Transdermal Patch formulation of Extract of *Acalypha indica* Linn. for P2 (85.24%), H4 (70.56%), and S7 (84.41%) at the end of 12 hours study.

The release behavior of drug from the patch formulation exhibited a biphasic pattern, which is characterised by faster onset of action and slower sustained release.

The data obtained from *in-vitro* release profile after 12 hours was fitted with various kinetic equations to determine the mechanism of drug release and release rate as indicated by higher correlation coefficients (r^2). The drug release from patch formulation follows zero order and non-fickian diffusion.

These findings indicates that the drug release from the formulated patch were diffusion controlled.

To confirm the release mechanism, the data of H4 (65.56%) release were applied to Korsmeyer peppas equation to find out the release exponent ‘n’, which indicates the mechanism of drug diffusion from the patch formulation. The data were fitted with equation as indicated by higher correlation coefficients (r^2) and mechanism was found to be non-fickian diffusion (anomalous transport).

The kinetic data of H4 formulation was best fitted to Korsmeyer peppa’s model and the value of regression coefficient, r = 0.984 and n = 0.83 which follows non-fickian, Super case-II transport.

From the results of *in-vitro* release and physicochemical studies, H4 (HPMC) was concluded as best formulation. So, H4 was subjected to further evaluation such as *ex- vivo* and stability studies.

The *ex-vivo* studies results showed 65.56% of drug release at the end of 12 hours. It concluded the sustained release property of polymer, through the skin

Stability study was performed for the period of three months. The physicochemical parameters like visual appearance, colour, texture and drug content and drug release were studied. The results showed that there is no significant change from its initial nature till the period of three months at 40˚C ±2˚C/75± 5% RH. Also the screening anti microbial studies confirmed the antimicrobial activity of the formulation.

The present work has achieved the objectives of formulation of Transdermal patch of Methanolic Extract of *Acalypha indica* Linn. by using different polymers like Pectin, Sodium alginate and HPMC. It showed appreciable amount of permeation through the skin (*ex-vivo*) and sustained release for the period of 12 hours. The release kinetics confirms that the formulation followed zero order, non-fickian diffusion model.

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